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Genetic recombination and spatial chromosome relations in maize

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Genetic recombination and spatial chromosome relations in maize

by

Ming-Hung Yu

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INTRODUCTION

The tendency of parental combinations to remain together is termed linkage; genes show linkage because they are on the same chromosome. When new combinations of linked genes occur they are referred to as recombination events. Genetic loci are arranged in a linear order, and recombination can occur within a gene as well as between genes on the same chromosome.

Within a genome there is a wide diversity in recombination among chromosomes. Crossover frequency per unit of physical distance varies throughout the chromosome, because the centromere exerts a marked influence on the rate of crossing over in its neighborhood. In order to investigate variation in recombination throughout the maize genome a standard marker, waxy locus, in maize has been relocated to various positions in this study.

The gene, the unit of function, can be subdivided into many linearly arranged structural elements. This has been demonstrated for numerous loci among microorganisms. With most higher organisms, however, it is difficult to determine whether the functional unit is subdivisible by recombination. The difficulty is at least partly due to the technique of handling, collecting and screening sufficiently large number of progeny to detect rare recombination events.

In maize, a higher plant, intracistron mapping has been demonstrated by Nelson (1959, 1962) with a sensitivity

approaching that available in microorganisms. This is based on the differential staining reaction of starch in pollen of different genotypes. It is possible to use the pollen grains as the unit of observation in genetical investigations on the waxy locus, because the starch composition of a haploid pollen grain is determined by its own genotype (Demerec, 1924; Brink and MacGillivray, 1924). Pollen grains with waxy (wx) alleles stain reddish brown in iodine-potassium iodide solution; whereas non-waxy (Wx) pollen stains dark blue. Therefore, in wx plants genetic recombination at this locus can be demonstrated by the appearance of dark blue staining pollen (Wx). This technique has a distinct advantage in genetic investigation with maize in that large populations of hundreds of thousands of genotypes (pollen grains) can be obtained and scored relatively easily.

Intragenic recombination can be determined from the possible single crosses (F_1) among a given number of different wx alleles. If the frequency of Wx pollen grains from the F_1 plant is significantly higher than the mean of the Wx frequencies in the parental stocks, it is indicative of genetic recombination between two mutant sites of the wx locus. This frequency also reflects the distance between different mutant sites. Nelson (1968) has studied an extensive series of crosses from the independently originating wx mutants on the standard chromosome 9.

It has been known that recombination frequency between

two genes is determined by the position of the genes in relation to the centromere and not the specific nature of the section of chromosome itself (Beadle, 1932; Graubard, 1932). The frequency of crossing over increases as one approaches the middle of the chromosome arm, and is greatest in the distal half of the chromosome. However, the effect on recombination frequency in changing the position of the wx locus from the centromere in a maize genome has never been determined.

This study is concerned with whether intragenic recombination at a given locus is affected by changes in position of this locus in the genome. What effects will result when a gene locus is relocated at different translocated positions. In this instance, the wx locus is used as a tool to investigate the diversity of recombination potential in different parts of the maize genome.

Two types of translocations are used to influence the wx locus. Five chromosome interchanges with proximal breakage points are used to move the wx locus to various positions away from the centromere (other than chromosome 9). Five other translocations with breakage points distal to the wx locus are used to lengthen the short arm of chromosome 9 with a segment from another chromosome of the same genome.

LITERATURE REVIEW

In surveying the literature pertinent to this study, it became apparent that this review could best be handled by separating it into four parts. The first part pertains to genetic studies on the diversity in exchange potential within a genome. Although the author considers all of these studies to be intergenic, relatively few of the previous studies were designed to study intragenic recombinations, especially in higher organisms. Part two deals with the phenomenon of intragenic recombination in microorganisms. This knowledge is important because genes in all organisms from viruses to higher plants or mammals are complex in structure and basically similar in composition (Lewis, 1967). The third part relates to intragenic studies in the wx locus which were utilized in obtaining the experimental results. The last part of this review deals with chemistry of the waxy phenotype. The interaction of iodine solution with pollen grain starch is the basis for the data on recombination frequencies in this study.

Diversity in Exchange Potential Within a Genome

Genes on chromosome

The concept of the gene is a logical extension of the hereditary character it determines (Swanson, 1957). For the existence of a gene can be recognized only when the mutated form can be compared, in a variety of ways, with the normal

or wild type gene from which it presumably arose. The genes are arranged in a linear sequence along the chromosome (Sturtevant, 1913). From linkage and the process of crossing over, the gene can be referred to a particular region of a chromosome.

In fact, a gene is not an indivisible unit of heredity; it is composed of a continuous sequence of nucleotides that determines the sequence of amino acids in protein, e.g., tryptophan synthetase (Yanofsky et al., 1964, 1967). An alteration can occur anywhere in this chain of nucleotides, giving rise to different proteins and hence to various modifications of the same basic phenotype. When this occurs, the change is recognized as a mutation. Recombination can occur between two mutant alleles that affect the same function just as recombination can occur between two genes that affect different functions (Levine, 1968). All genes have a very large number of sites at which mutation can occur, and these mutable sites are arranged in a strictly linear order. Thus, a gene is a discrete chromosome region which (a) is responsible for a specific cellular product, and (b) consists of a linear collection of potentially mutable units (mutable sites), each of which can exist in several alternative forms and between which crossing over can occur (Watson, 1965).

Crossing over and the centromere effect

The precise time at which crossing over takes place is a controversial matter, but it most likely occurs at the mid-prophase of meiosis, i.e., at or near pachynema, and by a breakage-joining mechanism (Rhoades, 1968; Peacock, 1971). This is shortly after reduplication of the chromonemata that gives rise to two new chromatids. The early studies (Bridges, 1916; Anderson, 1925) of the chromosome theory of inheritance clearly demonstrated that crossing over occurred in the four-stranded stage of meiotic prophase. All four chromatids of a tetrad can participate in crossing over as shown by the fact that multiple-strand double crossover occurred (Lindegren and Lindegren, 1937). Crossing over was preceded by a synapsis of homologous chromosomes, and that by a reciprocal exchange of chromatin material it led to the breaking up of linkage groups; exact reciprocal products are usually formed (Rhoades, 1961). Blocks of genes rather than single loci can be exchanged whenever a crossover occurred.

In maize, as in Drosophila, it has been possible through genetic studies to demonstrate that the chromosomes are divided into chromatids at the time crossing over occurs (Rhoades, 1933). In addition to the genetic proof it has also been possible in maize to show that crossing over takes place between chromatids by direct cytological observations (McClintock, 1931; Creighton and McClintock, 1932).

The linear order of genes is the same for cytological

and genetical maps, but physical and genetical map distances are not interchangeable units of measurement. A comparison of genetical and cytological maps shows that exchange frequency is not uniform throughout the chromosome. The frequency of crossing over will be more frequent in the middle region of the chromosome arm and less frequent in the proximal and distal regions (Stephens, 1961a). In Drosophila, for example, a comparison of the cytological and genetical maps indicates that crossing over per unit of physical length near the centromere is less frequent than in distal regions (Beadle, 1932).

There is striking evidence in maize that crossing over in distal segments is much more frequent per unit of physical length than in more proximally located regions. According to McClintock (1941) and Rhoades (1955) the short arm of chromosome 9 has about 20 chromomeres. The proximal one-third is characterized by large, deep-staining, closely packed chromomeres which have been likened to the heterochromatin of Drosophila chromosomes. The distal two-thirds of the short arm is euchromatic because it is composed of small, widely spaced chromomeres. The total length of the genetic map of the short arm is between 60 and 70 units. The wx locus lies approximately in the middle of the short arm of the pachytene chromosome. The amount of recombination between wx and centromere, which is a segment comprising about one-half of the entire arm, is no more than 12 map units. Thus one-half of the physical length is involved with one-sixth (12/70) of the

total exchange frequency of the short arm of chromosome 9. On the other hand, the distal half of the arm extending from the wx locus to that of Dt has a map length of 59 units (Rhoades, 1945, 1955). The yg_2 locus is situated in the terminal chromomere of the short arm (McClintock, 1944). Disregarding the heteropycnotic terminal knob, the only part of the short arm distal to yg_2 is the threadlike strand which connects the ultimate chromomere and the knob. Although physically very near one another, the Dt and yg_2 loci are 7 map units apart.

The reduction in crossing over frequency in the neighborhood of centromere has been attributed to the influence of the centromere. In the Drosophila experiments Beadle (1932) made use of a translocation involving the III and IV chromosomes, which was subsequently studied in the homozygous state. He showed that the low frequency of crossing over depends on the proximity of the region to the centromere itself. That is, crossing over in a genetic interval can be decreased or increased by translocation to a position nearer to or farther from the centromere respectively. Graubard (1932, 1934), through the use of an inversion, confirmed Beadle's results, making it quite clear that it is the position of the genes and not the genes themselves, that determine the frequency of crossing over.

In the large autosomes of D. melanogaster, Thompson (1964) reported that the centromere effect, an inhibition of centric

crossing over in normal females, not only depends on the pairing of homologous centromeres, but also requires that their pairing be in the same plane as the pairing of adjacent centric regions. With translocations or pericentric inversions having a basal break, real and quantitatively significant increases in crossing over are observed in the region just distal to the basal breakpoint. On the basis of these studies, Thompson suggested that the pairing of homologous centromeres initiates a repulsion of those same centromeres before the time of crossing over.

Compensating exchange with a genome

The number of chiasmata per nucleus tends to remain constant. Increase in chiasmata frequency in one part of the genome tends to be compensated elsewhere (Mather and Lamm, 1935). Sturtevant (1919) had early pointed out that a decrease in crossing over between one pair of homologues, due to the presence of a heterozygous inversion, increased the frequency of crossing over in nonhomologous and unrelated pairs of chromosomes in the same cell. It appeared, therefore, as if a certain amount of potential energy were available for crossing over within each meiotic cell. If one pair of homologues did not utilize its alloted amount, other pairs could draw from the reserve to increase their frequencies of crossing over.

However, there remains a strong possibility that the interchromosomal effects on crossing over are not actually increases

per se, but are due rather to the elimination of low crossover chromosomes (Cooper et al., 1955). This phenomenon has been further interpreted by Grell (1962) as "distributive pairing". In female Drosophila meiotic chromosomes which for any reason fail to undergo exchange participate in distributive pairing. This pairing is not restricted to homologues but may also involve nonhomologues. When nonhomologues pair distributively, abnormal patterns of chromosome distribution are found, that is, nondisjunction of homologues and regular segregation of nonhomologues (Grell, 1967). As a consequence, this leads to a reduction in the number of recombinations of the heteromorphic chromosome pairs.

The number of chiasmata per bivalent also is rather strictly limited and probably under genetic control (Stephens, 1961a). Stebbins (1950) and Grant (1958) suggested strongly that chromosome number and chiasmata frequency per bivalent have been built into an organized system in each species as a result of natural selection.

An early study shows that the rearrangement of a chromosome, when existing in a homozygous condition, shows no reduction in crossing over. In Drosophila females homozygous for inversions show about the same amount of crossing over as do homozygous normals (Sturtevant and Beadle, 1936). This is also in agreement with the conclusions of Beadle (1932) that the distribution of this crossing over is altered by its relation to the centromere, a given section giving less crossing

over if it is near the centromere, more if it is near the free end of the chromosome.

When a chromosomal rearrangement is in a heterozygous condition, it would be expected not only to alter the linear order of genes in the affected chromosome, but also to alter the frequency of crossing over between it and its normal homologue. There is evidence in maize which can be interpreted as compensation in the proximal region of a chromosome for restricted recombination in a more distal region. Rhoades and Dempsey (1953) found that crossing over within the loop of an inversion was usually genetically ineffective, owing to the dicentric formation. But in the immediate proximal segment the loss in recombination was about exactly compensated. A similar situation was found by Morgan (1950) and Russell and Burnham (1950) working with different inversions. Recently, Maguire (1969) found that a proximal crossing over increase was somehow attributed to distal crossing over inhibition.

Stephens (1961b) studied recombination between supposed homozygous chromosomes of the two tetraploid species Gossypium barbadense and G. hirsutum and reported a reduction in the Yg_2-r_2 region from 20.7% in pure G. hirsutum to 8% in the interspecies hybrid. He further showed that there was a compensatory increase in recombination in the remaining part of the chromosome though not in the immediate neighborhood as in corn, leaving the total map distance unalternated.

In Drosophila crossing over frequency in the presence of

heterozygous translocation is decreased markedly in the broken arm, especially in regions adjacent to the break point and increased slightly in the opposite arm (Dobzhansky, 1930). Using a variety of translocations, Dobzhansky (1931) has shown that the greatest reduction in crossing over is in the neighborhood of the breaks, and that the reduction becomes less pronounced as sections of the chromosome more remote from the points of breakage are tested. The decrease in recombination adjacent to the point of translocation has been interpreted as due to incomplete synapsis, resulting from pairing conflicts, a conflict of attraction forces or competition in pairing (Dobzhansky, 1931; Dobzhansky and Sturtevant, 1931), and might be expected regardless of the position of the break.

Burnham (1962) related the effect on recombination to nonhomologous pairing which occurs when the pachytene "cross" is not at its true position. Burnham reported that in maize interchange heterozygote T5-9a (5L.7-9S.3) there was considerable reduction in crossing over, with the greatest effect in the $\underline{sh}_1$-$\underline{wx}$ region, less in the $\underline{yg}_2$-$\underline{sh}_1$ region. There was no reduction in any of the other regions. At pachytene the "cross" was at various positions in the short arm of chromosome 9. Nonhomologous pairing appears to explain the reduction.

Anderson et al. (1955a) found that in maize interchanges involving breaks near the centromere in chromosomes 4 and 6 translocation heterozygotes show very little reduction in crossing over near the breaks and they suggested that there

is an appreciable region of undetermined length between the centromere and the $\underline{su_1}$ locus in which there is little recombination in the normal stocks and that interchanges in this region therefore have little effect on recombination. The same authors (1955b) also found that interchanges between Y and Pl caused a reduction in recombination between Y and Pl from a normal value of about 31% to less than 10%. This result also was obtained in Phillips' (1969a, 1969b) studies and can be extended to include $\underline{su_2}$.

Cytological studies of synapsis at pachynema in translocation complexes have afforded an adequate explanation of the reduction in crossing over at regions adjacent to the breakage point. In many pachytene figures it was observed that homologous regions were loosely paired at the translocation points. Since an intimate association of homologous regions is a necessary prerequisite for crossing over, the loose pairing often found near translocation points should result in a decrease in crossing over in regions adjacent to the breakage points. Pairing and consequently crossing over is nearly normal in those segments distantly removed from the translocation points. Burnham (1932, 1934) and McClintock (Rhoades, 1955) have shown that the position of the "cross" is not constant. Any shifting of the position of the "cross" from the true position involves the pairing of nonhomologous regions, even when they are intimately paired. Thus the occurrence of a considerable amount of nonhomologous synapsis should decrease

the frequency of crossing over. Studies with other translocations have shown decreases in crossover frequencies which are undoubtedly caused either by the failure of pairing near the center of the cross or by nonhomologous association or both.

Burnham (1962) summarized the effects of heterozygous translocations on crossing over in Drosophila based on information included in the descriptions of mutants by Bridges and Brehme (1944), and on data from Dobzhansky and Sturtevant (1931), Brown (1940) and Pipkin (1940). When both breaks were near the centromere, and consequently both interstitial segments were short, all showed nearly normal crossing over in both chromosomes. This is in agreement with Thompson's (1964) hypothesis. When the breaks were distal, recombination was greatly reduced near the breaks. When the break was near the middle of an arm, crossing over was reduced in the entire arm but greatest in the regions nearest the break. When the break was still closer to the distal end, crossing over was greatly reduced near the breaks but rose to nearly normal in regions farthest away, even in the same arm. This suggests that variable pairing accounts for most of the reduction.

Several hypotheses have been put forward to explain the interchromosomal influence of additional chromatin, deficiencies and chromosome rearrangements. In general, these hypotheses invoke mechanical perturbation of synapsis, physiological influences, or a combination of both (Nel, 1971). However, the mechanisms by which the interchromosomal effects are produced

remain obscure at the present time. Besides being affected by structural changes, recombination frequencies are also subject to the influence of several types of genes, which differ in respect of: the size of their effects, the extent of the genome affected, and the manner in which they act upon the processes involved in crossing over.

Other factors affecting crossing over

It was recognized early that crossing over was a variable phenomenon, subject to change by a number of environmental factors. Genetic maps, therefore, should be based on crossing over that takes place under certain specific conditions.

Among the intrinsic factors affecting the frequency of crossing over are age, sex and genotype (Bridges, 1915; Whittinghill, 1937; Hollander, 1938). Temperature is an obvious modifying extrinsic influence (Plough, 1917; Stern, 1926). In organisms in which crossing over is regularly found in both sexes, e.g., in plants or animals, the meiotic process does not necessarily produce the same percentage of recombinants in male and female germ cells. The frequency of recombination between any two genes may be (a) identical in the two sexes (or in the anthers and ovaries of dioecious plants), as in the garden pea; (b) higher in females than in males, as in the mouse, man, and the rats; or (c) higher in males than females, as in pigeon and maize.

In maize, the frequency of recombination in a particular chromosomal region is usually higher in microsporogenesis than

in megasporogenesis, if a difference exists at all. The $\underline{C_1}$-$\underline{sh_1}$, $\underline{sh_1}$-$\underline{gl_{15}}$, and $\underline{wx^{Coe}}$-$\underline{wx^{90}}$ regions in chromosome 9 consistently had a higher recombination value when the heterozygous combination was used as the male parent (Nelson, 1964, 1966). Rhoades (1941) reported that crossing over in the $\underline{A_2}$-$\underline{Bt}$, $\underline{A_2}$-$\underline{Bm}$, $\underline{Bt}$-$\underline{Pr}$, and $\underline{Bm}$-$\underline{Pr}$ regions of chromosome 5 in maize was significantly higher in the male flowers than in the female. Since these four regions were adjacent to the centromere, it was suggested that differences in crossing over in male and female flowers might be confined to regions near the centromere. Unpublished data by Burnham (Rhoades, 1955) on crossing over in proximal regions of several chromosomes also show higher recombination values in male than in female flowers.

Two explanations have been proposed to account for the lower exchange values among females (Rhoades, 1941, 1955); namely, (a) there is a basic difference in rates of crossing over in mega- and microsporocytes, or (b) selective orientation of the tetrad on meiotic spindles could lead to the noncrossover chromatids being preferentially segregated to the basal megaspore. If the latter alternative were true, crossing over could be equally frequent in both kinds of flowers, but the observed amount would be less in the female gametes.

Pericentric inversion 5a (5S near centromere, 5L.50) in maize was studied by Morgan (1950) who reported 12.5% ovule abortion and 28.3% pollen abortion. The difference in abortion percentages were attributed to higher crossing over in the male

flower, which is in agreement with the genetic results of Rhoades (1941) for chromosome 5.

Hanson (1969) tested B-chromosomes in maize for their effects upon crossing over in the A-chromosomes 3 and 9. In both instances, crossing over was increased primarily in the double crossing over classes. B-chromosomes were shown to increase the interfered frequency of the singles. The overall effect of an increase in B-chromosomes was an additive increase in crossing over and general decrease in chromosome interference.

Nel's (1971) investigation shows that B-chromosomes, abnormal 10 (K10), and the as and el genes in maize have marked effects on recombination in particular regions. However, no generalizations can be drawn that will hold for all members of the maize component. Not only do different chromosomes vary in their response to these factors but, within a single chromosome, strikingly different recombinations are found in proximal as compared to distal segments.

The Phenomenon of Intragenic Recombination

Genetic fine structure in microorganisms

For many years it was generally thought that crossing over occurred between genes, not within genes themselves. The chromosome was viewed as a linear collection of genes held together by some nongenetic material, somewhat like a string of pearls (Belling, 1933). In the last two decades it has

been realized that all crossing over may occur by breakage and reunion of the genetic molecules themselves. The fact is that crossing over between two regions is much easier to detect if the regions are far apart on a chromosome. If they are very close, recombination is extremely rare and can be detected only by examining a very large number of progeny, too large to make a study of intragenic recombination practicable when genetic work was restricted to most of the higher organisms.

From microbial genetic studies it became evident that a gene is further subdivisible. Benzer (1955, 1956, 1957) proposed that recombination can take place within a gene simply because functional genetic units are composed of smaller recombinational and mutational elements. His work with rII mutants of bacteriophage T4 provided evidence that the organization of genetic material in bacteriophage is similar to that observed in bacteria and fungi. Moreover, it furnished the basis for a model of a gene locus, in which the sites of different recombining allelic mutants correspond to a few or even one nucleotide pair, in accordance with the Watson-Crick model (Watson and Crick, 1953) of the DNA molecule.

Benzer found that certain rII mutants gave no detectable wild-type recombinants with any of several other mutants, which nevertheless gave wild-type recombinants with each other. Such mutants can be represented as occupying a segment of the linkage map rather than a point. It was significant that back

mutation of such mutants had never been observed, whereas some of the point mutations showed reversion to wild-type not infrequently. The most likely explanation for both the recombination behavior and the stability of these mutants appear to be that they had originated through the loss of a segment of the hereditary material (deficiency) covering the length which they appear to occupy on the linkage map.

From the results of his overlapping deletion studies Benzer (1959, 1961) suggested that the functional unit of hereditary material, defined by a comparison of the phenotype of the "cis" and "trans" configuration of a pair of mutations, be called a "cistron". On the basis of his definition, a cistron would be a segment of the hereditary material within which the cis configuration produced a normal phenotype, and the trans configuration produced a mutant phenotype. It would seem, even down to its smallest molecular components, a cistron may indeed be analogous to the linear order in which the genes are integrated in the chromosome. His results permit representation of the mutation as alteration in a linear structure in which the functional units defined by the cis-trans test correspond to unique segments.

Gene conversion - nonreciprocal recombination

The theories of recombination have usually been concerned with reciprocal exchange between homologous DNA molecules to yield DNA molecules having a nucleotide sequence partly that

of one parent molecule and partly that of the other (Meselson, 1967). However, from numerous experiments it has been demonstrated that exchange events need not always lead to the production of reciprocal products. These nonreciprocal events had been called "gene conversion" by Winkler in 1930 (Roman, 1963). Gene conversion represents a departure from the expected intra-ascal 2:2 segregation (in fungal asci) of a single heterozygous site.

In fungi, it is possible to examine directly the four haploid meiotic products. The aberrant segregation was first clearly demonstrated in yeast by Lindegren (1953). Since then gene conversion has been demonstrated in a number of fungi both at meiosis and mitosis. The modern study of conversion began with Lindegren (1953, 1955) and Mitchell (1955) in Saccharomyces and Neurospora respectively, although the nonreciprocal segregation had been observed earlier. They demonstrated that a very short region may have a 3:1 ratio of parent types, with the normal (reciprocal) 2:2 ratio reestablished at closely linked marker loci on both sides. Gene conversion may also occur in higher organisms such as Drosophila. Evidence has been presented that all intragenic exchange events at maroon-like cistron (Smith et al., 1970a, 1970b) and a portion of the recombination events within rosy cistron in Drosophila (Chovnick et al., 1970; Ballantyne and Chovnick, 1971) occurred by way of a conversion mechanism.

Gene conversion could occur in both the mitotic and

meiotic phases of the life cycle; however, meiotic frequencies were at least 3 or 4 orders of magnitude higher than mitotic frequencies (Roman, 1963). Moreover, recent evidence strongly suggests that gene conversion is independent of major DNA synthesis, since it may occur both prior or subsequent to the major round of DNA replication in mitosis or meiosis (Fogel et al., 1971).

Conversion frequently extends over a short region rather than being confined to a single site, i.e., often to be correlated with the exchange of flanking markers. An intimate connection between conversion and reciprocal recombination is suggested by the fact that normally segregating markers on opposite sides of converted regions are very often recombined (Meselson, 1967). Genetic studies also indicated that, among tetrads selected for recombination between very close markers, the frequency of conversion is very high. Meselson concluded that the conversion process occurs in the immediate vicinity of at least a large fraction of all exchanges.

According to Fogel and Hurst (1967) evaluation of reciprocal and nonreciprocal aspects of recombination requires a genetic system with the following attributes: (a) all the meiotic products must be available, and isolatable as tetrads, and (b) closely linked markers flanking the locus within which recombination is being studied must be present. The outside markers allow determination of chromatid involvement in crossing over, (c) a substantial sample size of recombinant

tetrads must be available.

Analyses of allelic recombination have demonstrated the phenomenon of polarized recombination, and have led to the suggestion that recombination events are discontinuously distributed (Murray, 1969). However, the mechanism of gene conversion and its relationship to linked recombination is not yet understood.

Current models of genetic recombination, hybrid-DNA-correction models (Whitehouse, 1963; Hastings and Whitehouse, 1964; and Holliday, 1964), assume that formation of a heterzygotic region of DNA is a prerequisite for gene conversion and crossing over. At a mutant site in this heterozygotic region there can exist some mispaired bases, a condition assumed to be unstable. This may be corrected by enzymic replacement of the abnormally paired bases by new ones with normal hydrogen bonding. Depending on the direction of such correction, the wild type or mutant condition of the chromatid is restored. Processes of this type lead to marker segregation in the ratios 2:6 and 6:2. If the correction occurs in only one of the two chromatids with heterochromatic regions of DNA, postmeiotic segregation (3:5 or 5:3) is observed.

Fogel and Mortimer (1969) considered that gene conversion is a process of informational transfer (or co-conversion), which frequently is allele-specific rather than locus-specific. Approximately 2% of the haploid genomes are replaced in each meiosis by information contained in the homologous nonsister

chromatids. The replacement involves randomly located segments of which modal length is of the order of some hundreds of nucleotides. In this way, conversion provides a mechanism for rapid meiotic rearrangement of genetic variability. It is not restricted by positive interference and results in the oscillation of relative short information segment, when compared with crossing over. When alleles are widely separated they behave as essentially independent units in conversion (Fogel et al., 1971).

Recombination and polarity

Intragenic recombination is usually polarized, i.e., within a gene or a part of it, alleles situated proximally to one end of this region convert in two-point crosses more frequently than those situated distally (Gajewski et al., 1968). According to the models mentioned above, polarization in intragenic recombination results basically from the unequal changes of various mutant sites to be involved in the heterozygotic region of DNA. Furthermore, the mutations themselves may influence this chance.

The pattern of polarity, i.e., direction and degree, is better described by a function rather than a model. The explanations of polarized allelic recombination require either that recombinational events are discontinuously distributed (e.g., models of Holliday's, etc.), or a component of the recombination process is polarized either within small subunits

(polarons) or with respect to the gross organization of the chromosome. Lissouba et al. (1962) studying two-point crosses involving a series of allelic spore color mutants in Ascobolus, found that in given crosses recombination "always" resulted from conversion of the same mutant parent. Conversion showed a consistent polarity according to their recombination map of these mutants.

Where a clear polarity is found it does not necessarily remain constant in direction throughout a gene (Fincham, 1970). Murray (1969, 1970) demonstrated that the direction of the polarity of allelic recombination changes is a function of the position of sites within a gene. For each of these regions the polarity is such that the sites of preferential conversion are located toward the end of the gene. This might explain why certain polarities are in a definite direction.

The recombination between the methA mutants of Aspergillus nidulans is reported to be very strongly polarized (Putrament et al., 1971). The mutants can be mapped in respect to each other and in respect to the flanking markers, both on the basis of the relative frequency of one of the P classes and one of the R classes. In all crosses it is the distal mutant which converts predominantly.

It appears that, in most of the studies where many alleles were used, polarization was only tentative, in some loci it was not at all, or its pattern was very complicated (Paszewski, 1970). It seems to be difficult to make any generalization

concerning this phenomenon when data from different loci are compared.

Association of conversion and crossing over

All investigators agree that gene conversion is highly correlated with crossing over between the same two strands as were involved in the conversion (Fincham, 1970). The associated crossing over occurs close to the conversion event. The degree of association varies somewhat from one case to another; it is often about 50 percent, but may be higher or lower. This means that conversion may occur without crossing over.

In Kitani and Olive's (1967) Sordaria analysis crossing over was associated with somewhat less than 50 percent of conversion events. Murray's (1969) data on various Neurospora me genes show considerable variation in this respect between genes; however, the association between conversion and crossing over was clearly significant. In the rosy cistron of D. melanogaster (Chovnick et al., 1970; Ballantyne and Chovnick, 1971) an analysis of exceptional ry^+ survivors has shown that approximately 50 percent of the events leading to the ry^+ half-tetrads are classical recombination, and the others, conversion. In yeast, intergenic and intragenic (mainly nonreciprocal) recombinations were influenced in a similar way by ultraviolet radiation or by X-rays (Esposito, 1968).

It is apparent that about half the conversion in all

categories are associated with reciprocal recombination of the adjacent outside markers, i.e., that two conversions are equivalent to one resulting from crossing over (Fogel et al., 1971). The quantitative relationship between crossing over and conversion prevails regardless of the interval length in which outside marker recombination is scored. It is supposed that events leading to recombination must occur very close to the converted segment or that conversion and crossing over are simply expressions of the same fundamental event, the same primary event in DNA.

It was suggested that every cross-over event is associated with conversion over a shorter or longer length of a chromosome. With increasing distance of mutant sites within a gene, the relative frequency of crossing over is usually increased (Paszewski, 1970). Gene conversion, like crossing over, occurs in both chromatids of a chromosome at random. There is a sequence of events leading to gene conversion which is necessary but insufficient to accomplish crossing over.

Intragenic Recombination at the wx Locus

Methods of investigation

The frequency of intragenic recombination events at a particular locus is normally determined by the genetic composition at that locus. In maize, the starch type of pollen grains is an expression of the genotype of the wx locus (Demerec, 1924; Brink and MacGillivray, 1924). The haploid

pollen grain could be used as the unit of observation in higher plants (Nelson, 1957). A method for analyzing the fine structure of the wx locus was developed by Nelson (1959, 1962) based on the differential staining reaction of starch in pollen of different genotypes. The nonwaxy (Wx) pollen contains starch which has approximately 25% amylose with the remainder being amylopectin and this stains blue-black in the iodine-potassium iodide solution. The waxy (wx) pollen contains starch which is mostly amylopectin and stains reddish-brown. Utilizing this method, the low frequency recombination events from a large population size, e.g., 10^4-10^5 pollen grains, could be obtained and scored with ease under a microscope.

More than 20 different naturally occurring wx mutants have been found. When two different mutants are crossed the frequency of recombination can be studied by the pollen grains of the F_1 cross. If the frequency of Wx pollen is significantly higher than that of either parental mutant, the mutant sites of the two alleles are interpreted to be spatially separated. This frequency is also expected to reflect the distance between one mutant site and the nearest point of another mutant, since most of the Wx pollen grains are results of intracistronic recombination. A revised map of the wx locus locating 24 alleles has been drawn by Nelson (1968) using the overlapping deletion method of Benzer (1959) and conventional genetical analyses. Recombination frequencies

across the locus are not additive in this map. It will be noted, however, that some Wx pollen grains might arise through back mutations, suppressor mutations, interallelic complementation and even contamination from wind-blown pollen.

Different explanations have been offered to explain the wx mutants. Deficiencies and inversions of varying sizes could account for mutations that appear to cover a segment of the locus (Nelson, 1968). However, Wx pollen would not be produced in the deficiency homozygotes or the F_1 hybrid of two mutants which have corresponding deletion chromosomal regions. Bianchi and Tomassini (1965) suggested that these wx alleles (e.g., C, 90, M-1A, etc.) are likely to be due to base substitution in the DNA polynucleotides. This idea was supported by Amano and Smith (1965) in treating seeds and young seedlings of I Sh Wx stock of maize (Zea mays) with an aqueous solution of ethyl methanesulfonate (EMS) where the ethyl group was responsible for alkylation of DNA bases, which was followed by biochemical processes leading to the ultimate mutation.

Factors affecting intragenic recombination

"Point mutations", or at least minor deletions at the wx locus have been induced at sites within the wx locus in maize by ethyl methanesulfonate (Neuffer and Ficsor, 1963; Briggs et al., 1965; Amano, 1968; and Briggs, 1968). Studying the recombination between wx alleles C and 90, Melnyczenko (1970) found that B-chromosomes increase intragenic

recombination at the wx locus. Whereas Briggs and Smith (1965) found that X-radiation at the meiosis stage has a significant effect on reducing recombination frequency at the wx locus.

With the use of chromosome interchanges, the wx locus on chromosome 9 has been relocated to various positions in the maize genome (Yu and Peterson, 1971). Four wx alleles, C, B, 90, and H21, were crossed to different translocation stocks. From the results of the available homozygous translocation, heteroallelic combination pollen analysis, it was found that, in most instances, the frequencies of wx intragenic recombination of the rearranged chromosomes were lower than that of the control irrespective of the direction of change with respect to the distance to the centromere. A significant difference in degree of reduction in recombination values is found for different heteroallelic combinations at the same location, and in one instance for the same heteroallelic combination at a different chromosome position.

Chemistry of the Waxy Phenotype

The components of maize starch

The waxy gene (designated as wx) has been of great importance to the genetics of maize because its presence is readily detected and because it is inherited in a simple Mendelian fashion (Kempton, 1919; Brink, 1929). When kernels are dried below a certain moisture content (about 13 percent)

the opaque appearance becomes evident (Andres, in Hixon and Brimhall, 1968). This was determined by utilizing the Steinlite Moisture Tester. It was found that the critical moisture level of the kernels was slightly more than 13.03% to show the opaque appearance from the average of four waxy mutants; e.g., $\underline{wx}^{C}$ = 13.07%, $\underline{wx}^{B}$ = 12.85%, $\underline{wx}^{90}$ = 13.00%, and $\underline{wx}^{H21}$ = 13.18% (Yu, unpublished data).

The starch content of endosperm as well as pollen grains of maize homozygous for the wx (waxy) allele are almost entirely amylopectin, a branched-chain polysaccharide (Sprague, Brimhall, and Hixon, 1943; Kramer and Whisler, 1949; Hixon and Brimhall, 1968). In contrast, seed or pollen which contains the Wx (nonwaxy) allele has approximately 25% amylose with the remainder being amylopectin in the starch (Senti and Dimler, 1959; Nelson and Rines, 1962).

Amylopectin has a branched structure in which chains containing an average of about 20-24 α-(1-4)-linked glucose residues are interlinked by α-(1-6)-glucosidic linkages to form a ramified or bush-like structure (Carter and Lee, 1971). Amylopectin is heterogeneous (different in degree of branches and sizes of molecules) and can be separated into a series of subfractions having a range of molecular weights.

Amylose, the minor component, is an essentially linear, or slightly branched polymer of glucose containing more than 99% of α-(1-4)-glucosidic linkages. Although the amylose content of most maize starches is 15-25% (Creech, 1968),

certain varieties of maize, e.g., amylomaize, may contain 50-75% of amylose (Vineyard et al., 1958), while waxy-maize starch contains less than 1%. It contains one non-reducing terminal glucose residue for every 200-350 glucose residues.

Electron-microscopy studies of the development of starch granules in maize endosperm have shown their appearance within four days of pollination (Manners, 1968). Those two components of the starch granule, both polymers of glucose, possess different molecular weights. The amylopectin component has a higher value of about 10^7-10^8, whereas the amylose component has a lower average of 10^5-10^6. Fractions intermediate between amylose and amylopectin have also been isolated in small yield from corn starch of high amylose content. These fractions have chain length of 30-47, and are less highly branched than amylopectin.

Starch synthesizing enzymes

That the waxy gene exerts its influence on starch synthesis through the media of specific enzymes is a logical hypothesis, though the enzymes concerned are not yet completely understood. Three plant enzymes are now known which can synthesize a linear chain of α-(1-4)-linked glucose residues (Manners, 1968). These enzymes, phosphorylase, the D-enzyme and starch UDP-glucosyl transferase, are transglucosylases which transfer D-glucose residues, usually singly, from a suitable donor substrate to an acceptor molecule which must

already contain a small number of α-(1-4) residues. A description of these enzymes follows (Manners, 1968):

(1) Phosphorylase (P-enzyme) It can synthesize amylose-type molecules from α-glucose-1-phosphate. The enzyme is present in largest quantity during the first stages of growth, 5.1 units 16 days after pollination, then rapidly decreases to 0.28 unit at kernel maturity.

(2) D-enzyme (disproportionating enzyme) This enzyme catalyses disproportionation reactions between maltosaccharides, e.g., it can transfer a maltosyl unit from one maltotriose molecule to another, giving maltopentaose and glucose. Also D-enzyme provides a system for the synthesis of low-molecular-weight amylose.

(3) Starch UDP-glucosyl transferase This enzyme catalyses the incorporation of glucose from UDPG (uridine diphosphate glucose); the transferred glucose units are attached by α-(1-4)-linkages, on the non-reducing ends of the polysaccharide acceptors. Both amylose and amylopectin components can serve as acceptor substrates. In the past decade the further development in this field showed that ADPG (adenosine diphosphate glucose) was always a more efficient glucosyl donor than UDPG.

However, it is noted that neither P-enzyme nor D-enzyme

(which normally produce linear chains from maltosaccharides) nor starch UDP-glucosyl transferase (which has a chain-lengthening action) are able to create new molecules, or to directly polymerize glucose-1-phosphate. The mode of synthesis of the various acceptor substrates remains uncertain.

In amylopectin molecules, chains of α-(1-4)-linked glucose residues are interlinked to form a highly branched structure, α-(1-6)-inter-chain linkages. The "branching enzyme", or the cross-linking enzyme, is called Q-enzyme (Peat, Bourne, and Barker, 1948).

Q-enzyme This enzyme has a purely synthetic function, involving a transglucosylation reaction. For the mechanism of Q-enzyme a chain of glucose residues is detached from the original substrate and transferred to an adjacent chain, where it is attached by α-(1-6)-linkage.

At the present time little is known about the specificity and mechanism of action of Q-enzyme in starch synthesis (Greenwood and Milne, 1968). Both P- and Q-enzymes occur in tissue with starches of varying amylose content, including the waxy and high-amylose varieties. What, then, is the mechanism which prevents a certain genetically fixed number of linear molecules from becoming branched? Several systems have been proposed, but at this stage, it is difficult to make a choice (Badenhuizen, 1969). According to Dr. D. French (personal communication), Q-enzyme is present and functioning completely in the Wx genotype. Another enzyme, ADPG pyrophosphorylase is

absent in wx; "somehow" absence of this enzyme prevents amylose formation.

The reaction of starch with iodine

Weatherwax (1922) found that wx endosperm turns red when tested with iodine. The reddish-brown staining starches are composed almost entirely of amylopectin. The blue-black staining starches contain both a linear polymer of glucose (amylose) and a branched polymer (amylopectin) in varying proportions, but amylose is entirely responsible for the blue-black iodine color. In aqueous solution the amylose molecule forms a random coil which consists of linear segments of helical structure. The helical segments are built of 2-20 helical turns, all of which contain 6-8 AGU (anhydroglucose units). Formation of H-bonds between the neighboring glucose units seems to be likely (Hollo and Szejtli, 1968).

The most striking characteristic of amylose is its ability to give an intense blue solution with iodine in the presence of iodide ions, and the structure of the complex is an amylose helix with the iodine atoms forming a linear chain within. In amylose-iodine complex one iodine molecule is bound per six glucose residues for infinitely dilute iodide solution. X-ray-diffraction measurements on the solid amylose-iodine complex confirm that the iodine atoms are located in the center of the helix. The nature of the amylose-iodine complex depends on the chain length of the amylose. The

absorbancy of the iodine complexes increases rapidly and linearly as the average DP (degree of polymerization) increases from 18 to 72, and this region is believed to represent the building up of stable resonating linear ions. Thus the shift of wave length towards blue with increase of the length of the amylose chain can be qualitatively explained (Foster, 1943).

According to Lippert (Hollo and Szejtli, 1968), the amylose-iodine complex is red in the $\overline{DP}$ 5-10, violet between 10 and 25, and blue above 25. The shortest amylose-type chain which gives a finite blue value contains at least 10 AGU. There is a strict proportionality between chain length and blue value within the series containing between 10 and 90 AGU per chain. With chains of more than 80 AGU this proportionality no longer holds.

MATERIALS, METHODS AND SYMBOLS

Gene Symbol and Definition of Terms

Allele or Element	Description or phenotype
Wx	-The nonwaxy starch producing allele; expressed in endosperm and pollen grains by staining blue black with I_2-KI solution.
wx	-The waxy starch producing allele; recessive to Wx; expressed in endosperm and pollen grains by staining red to brownish red with I_2-KI.
wx^x or wx^y	-One of the wx alleles specified; x (or y) as general term = C, B, 90, or H21.
N	-Normal chromosomal constitution in the genome.
T	-Translocation or chromosome interchange as contrasted to normal (N); the exchange of segments of chromosome 9 and another chromosome.
T1	-One of the translocations specified; 1 as general term = 1, 2, ..., 10.
T/N or N/T	-Translocation heterozygote; semisterility is shown in pollen and ear culture.

Definition of Terms

Homoallelic combination	-Homozygous for the specific wx allele, e.g., wx^x/wx^x or wx^y/wx^y.
Heteroallelic combination	-Heterozygous for the specific wx alleles, e.g., wx^x/wx^y.
Waxy heterozygote	-Heterozygous for the wx genotype, e.g., wx/Wx.
Standard chromosome	-Normal chromosome 9 in maize genome.

Proximal translocation	-Translocation with the breakage point proximal to the wx locus in the short arm of chromosome 9.
Distal translocation	-Translocation with the breakage point distal to the wx locus in the short arm of chromosome 9.
Homotranslocation	-Homozygous for the specific translocation, e.g., Ti/Ti.
wx-centromere distance	-Distance between the wx locus and centromere in the chromosome.
wx-breakage point distance	-Distance between the wx locus and breakage point in the translocation chromosome.

Source of Materials

The mutant alleles investigated were received from Dr. O. E. Nelson of Purdue University, Indiana. The four wx alleles were wx^C, wx^B, wx^{90} and wx^{H21}; hereafter referred to as C, B, 90 and H21 (general term wx^x, wx^y, etc.). Ten chromosome translocation stocks were chosen for this study, which were obtained from the Maize Genetics Cooperative, University of Illinois. Among the ten translocations five involve breakage points proximal to the wx locus, and the other five, distal to wx (general term Ti). The structure of the ten translocation chromosomes are shown in Table 1 and Figure 1. The translocation chromosomes will also be referred to hereafter as interchanges or chromosome interchanges.

All the materials mentioned had been prepared by Dr. P. A. Peterson before this investigation started. The plants were grown at Iowa State Research Center in those summers from

Table 1. The constitution of interchange chromosomes (micron)

Chromosome interchange 1	Mean position of breakpoint[a] 2	Approximate distances[b] 3	4	Length of chromosome 5
Proximal		wx-ct[c]	wx-bk[d]	
T1 8-9 5391	8L.07;9S.33	3.61	1.08	24.18
T2 5-9 4871	5L.71;9S.38	22.56	0.31	60.31
T3 5-9a	5L.69;9S.17	25.17	3.55	62.92
T4 6-9b	6L.10;9S.37	4.15	0.46	25.30
T5 4-9 8636	4L.94;9S.09	38.79	4.79	70.66
Distal		wx-dt[e]	wx-bk[d]	
T6 8-9 5300	8L.85;9S.43	5.89	0.46	39.86
T7 3-9f	3L.63;9S.69	19.77	4.48	53.75
T8 6-9 8439	6L.06;9S.73	39.73	5.10	73.70
T9 7-9 6482	7L.01;9S.97	42.93	8.80	79.90
T10 7-9 7074	7L.03;9S.80	39.61	6.18	73.59
Standard		wx-ct[c]	wx-dt[e]	
Chromosome 9		6.18	9.27	43.24

[a]Longley (1961).

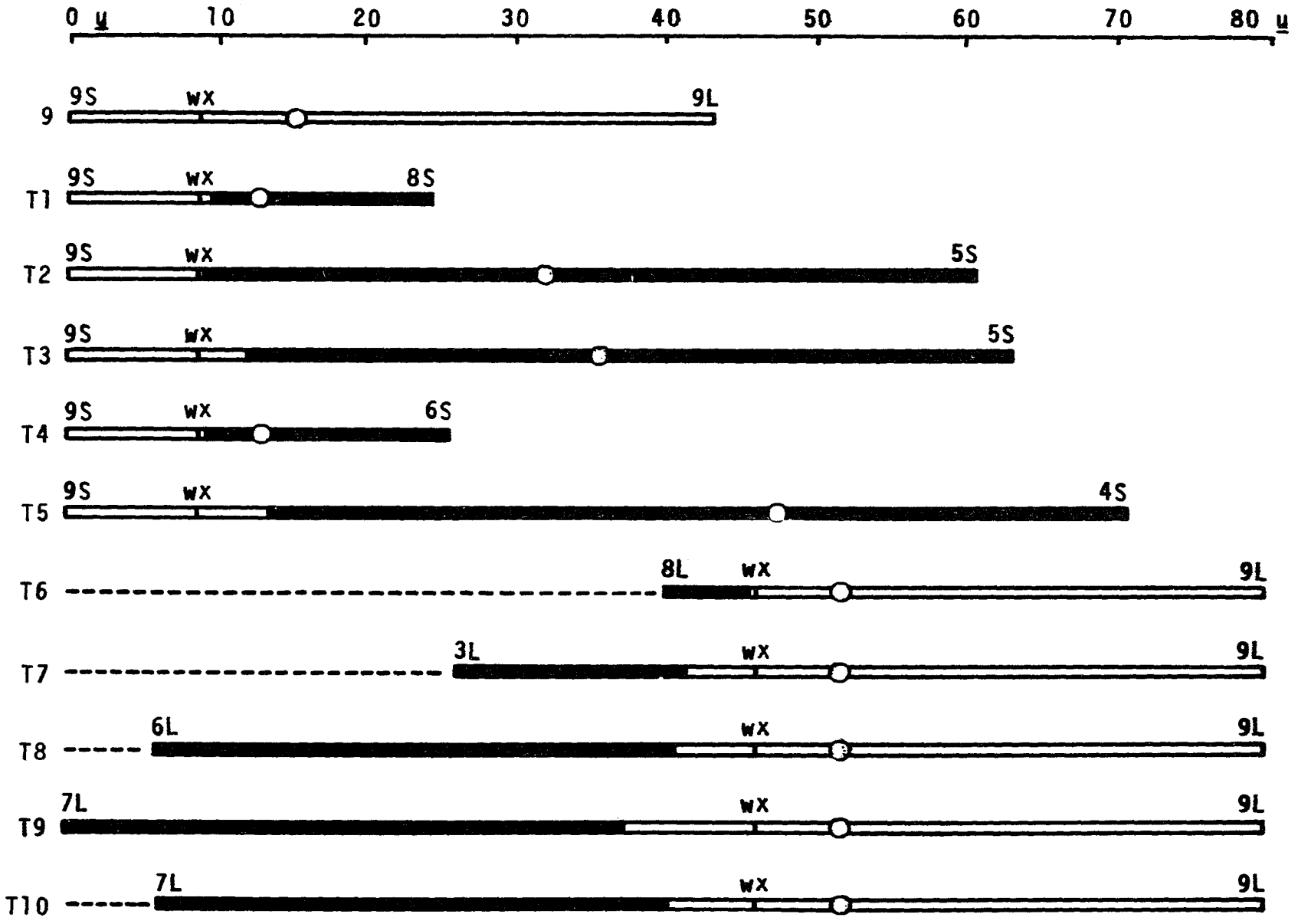
[b]Modified from Rhoades (1950).

[c]Waxy-centromere distance.

[d]Waxy-breakpoint distance.

[e]Waxy-distal end distance.

Figure 1. The standard and rearranged chromosomes; interchanges involve chromosome 9 of maize with breakage points proximal (T1-T5) and distal (T6-T10) to the wx locus (interchanges listed in Table 1)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4S
T6
8L
wx
9L
T7
3L
wx
9L
T8
6L
wx
9L
T9
7L
wx
9L
T10
7L
wx
9L

1967 to 1971, and in the Florida nursery as well as in the Agronomy Department's greenhouse in each winter from 1967 to 1970.

Development of the Waxy-Translocation Combinations

Step 1

The Wx translocation stocks, T1-Wx/T1-Wx, are crossed to the allele wx^X (N-wx^X/N-wx^X) on the normal (N) chromosome 9 in order to recover the heterozygous F_1 hybrids T1-Wx/N-wx^X.

$$\frac{\text{T1-Wx}}{\text{T1-Wx}} \times \frac{\text{N-}wx^X}{\text{N-}wx^X} \longrightarrow \frac{\text{T1-Wx}}{\text{N-}wx^X} \ (100\%).$$

The proximal translocation stocks (T1-T5) are crossed to the four wx alleles, C, B, 90 and H21; one distal stock (T6) is crossed to B and 90, and the other four (T7-T10), to alleles C and 90. All kernels from this cross are phenotypically non-waxy (Wx).

Step 2

The semisterile (SS) F_1 plants from cross 1, heterozygous for waxy and the interchange chromosome (T1-Wx/N-wx^X), are then backcrossed to the same parental wx alleles to recover the rare crossover chromosome strands T1-wx^X (Figure 1).

$$\frac{\text{T1-Wx}}{\text{N-}wx^X} \times \frac{\text{N-}wx^X}{\text{N-}wx^X} \longrightarrow \frac{\text{T1-}wx^X}{\text{N-}wx^X} \ (<1\%).$$

The proximal wx-linked interchange chromosome could be obtained if crossing over occurred between the Wx locus and the

breakage point (Figure 2a), and the distal one is obtained if crossing over occurred in the interstitial segment, distal to the Wx locus on chromosome 9^x (Figure 2b).

The resultant ear culture will include one-half waxy and one-half nonwaxy endosperm. The desirable Ti-$\underline{wx}^x$/N-$\underline{wx}^x$ combination is among the waxy ($\underline{wx}$) seeds. A large number of maize plants, e.g., 10^2-10^3 plants from $\underline{wx}$ progeny of step 2, are usually needed to procure a single desirable Ti-$\underline{wx}^x$ chromosome strand.

Step 3

The purpose of this step is to isolate the Ti-$\underline{wx}^x$ chromosome strand from the Ti-$\underline{wx}^x$/N-$\underline{wx}^x$ genotype. The field identified semisterile plant is crossed to an inbred line N-$\underline{Wx}$/N-$\underline{Wx}$ for this isolation.

$$\frac{\text{Ti-}\underline{wx}^x}{\text{N-}\underline{wx}^x} \times \frac{\text{N-}\underline{Wx}}{\text{N-}\underline{Wx}} \longrightarrow \frac{\text{Ti-}\underline{wx}^x}{\text{N-}\underline{Wx}} \ (50\%).$$

It is important that no $\underline{Wx}$ kernel is mixed among the $\underline{wx}$ seeds in the planting of this step. Misclassified $\underline{Wx}$ seeds will appear as semisterile plants; therefore, confirmation of the $\underline{wx}$ homozygotes among the selected plants is by examining the semisterile pollen with I_2-KI solution. All kernels resulting from this cross are expected to be of the non-waxy type.

Step 4

The desirable heteroallelic combination Ti-$\underline{wx}^x$/Ti-$\underline{wx}^y$ homozygous for the specific chromosome interchange is produced

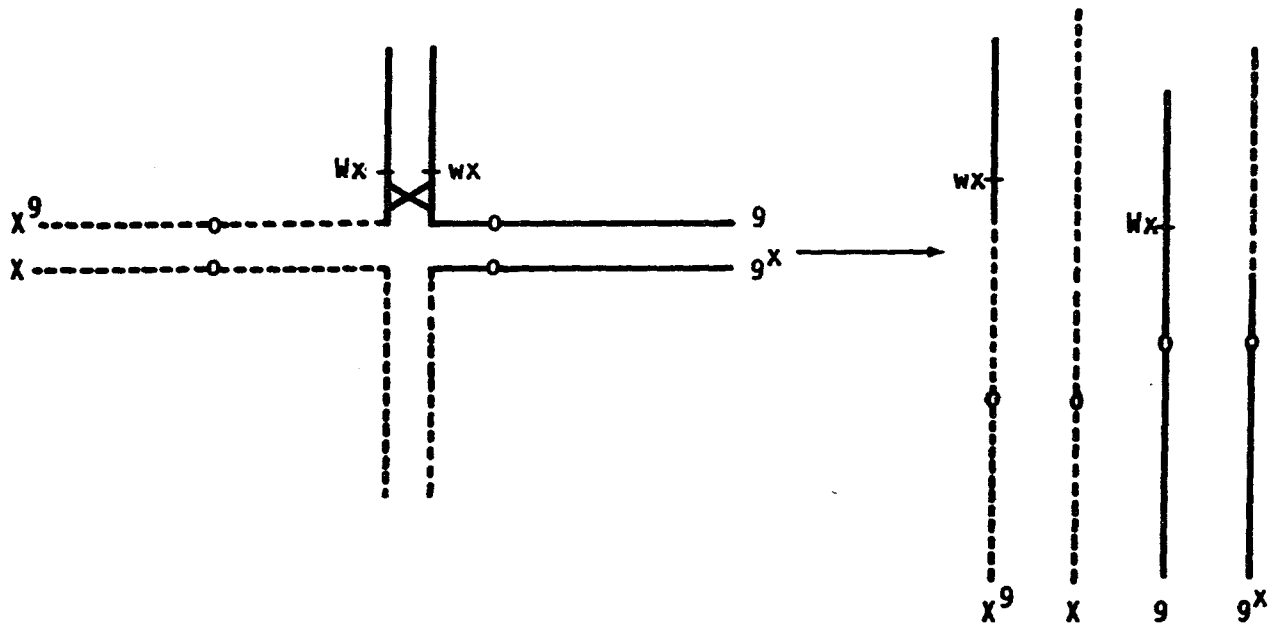


Figure 2a. The desirable (proximal) crossover events and recovered interchange chromosomes from the translocation heterozygote involving chromosome 9 of maize; 9 = standard chromosome 9 ; x = standard other chromosome; x^9 = interchange of x chromosome with segment from 9-x centromere (the arm length of chromosome 9 and the site of wx are not drawn to scale)

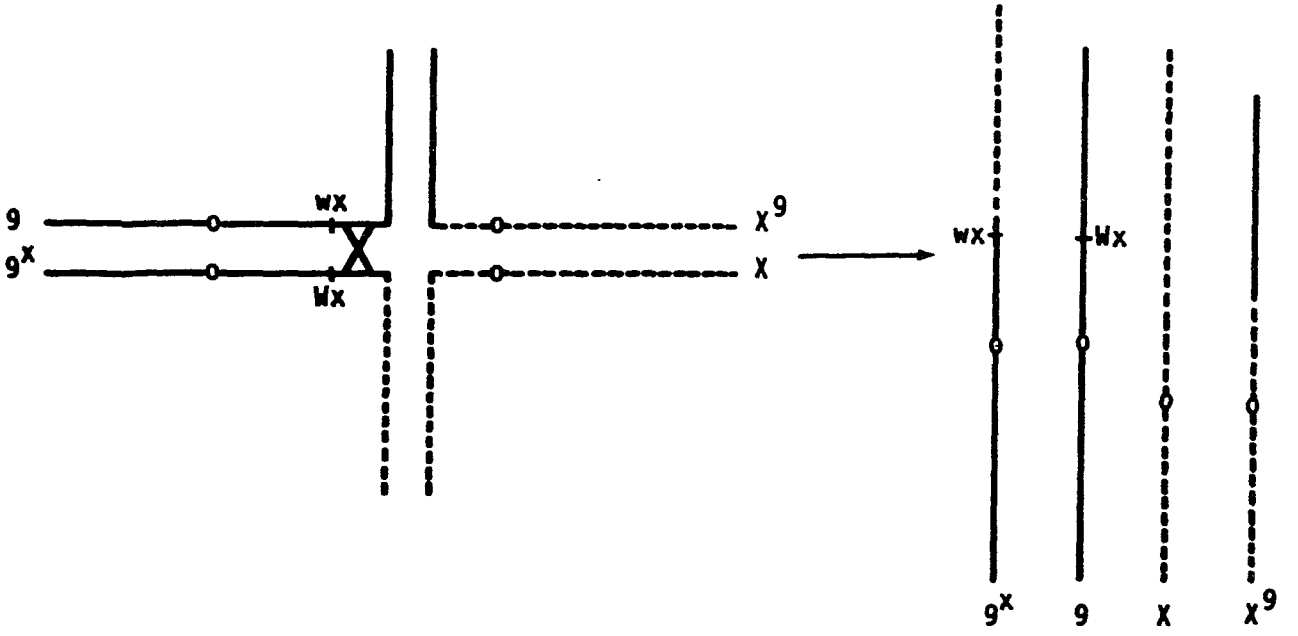


Figure 2b. The desirable (distal) crossover events and recovered interchange chromosomes from the translocation heterozygote involving chromosome 9 of maize; 9 = standard chromosome 9; x = standard other chromosome; 9^x = interchange of 9 with other chromosome-9 centromere (the arm length of chromosome 9 and the site of wx are not drawn to scale)

by intercrossing the appropriate semisterile plants from step 3.

$$\frac{\text{Ti-}\underline{wx}^{X}}{\text{N-}\underline{Wx}} \times \frac{\text{Ti-}\underline{wx}^{Y}}{\text{N-}\underline{Wx}} \longrightarrow \frac{\text{Ti-}\underline{wx}^{X}}{\text{Ti-}\underline{wx}^{Y}} \ (25\%).$$

All $\underline{wx}$ kernels (about 25%) are expected to be desirable.

The genotypes homozygous for both the $\underline{wx}$ allele and the interchange chromosome ($\text{Ti-}\underline{wx}^{X}/\text{Ti-}\underline{wx}^{X}$) are produced by self- or sib-crossing the semisterile plants within individual lines.

$$\frac{\text{Ti-}\underline{wx}^{X}}{\text{N-}\underline{Wx}} \times \frac{\text{Ti-}\underline{wx}^{X}}{\text{N-}\underline{Wx}} \longrightarrow \frac{\text{Ti-}\underline{wx}^{X}}{\text{Ti-}\underline{wx}^{X}}, \frac{\text{Ti-}\underline{wx}^{X}}{\text{N-}\underline{Wx}}, \frac{\text{N-}\underline{Wx}}{\text{N-}\underline{Wx}} \ (1:2:1).$$

Again, the $\underline{wx}$ kernels (about 25%) are desired. The $\underline{Wx}$ kernels (about 75%) from this homoallelic cross can be used for further proof of the translocation. Two-thirds of the $\underline{Wx}$ progeny plants should be semisterile, since the proportion of genotypes $\text{Ti-}\underline{wx}^{X}/\text{N-}\underline{Wx}$ and $\text{N-}\underline{Wx}/\text{N-}\underline{Wx}$ is 2:1.

Step 5

The desirable combination, heteroallelic or homoallelic for the $\underline{wx}$ allele and homozygous for the specific chromosome interchange, is obtained from the step 4 cross and represents the source of pollen for the recombination assay. From each heteroallelic line, at least two tassel-collected plants are outcrossing to normal (N) plants in order to confirm the existence of the translocation.

$$\frac{\text{Ti-}\underline{wx}^{X}}{\text{Ti-}\underline{wx}^{Y}} \times \frac{\text{N-}\underline{Wx}}{\text{N-}\underline{Wx}} \longrightarrow \frac{\text{Ti-}\underline{wx}}{\text{N-}\underline{Wx}} \ (100\%).$$

All the progeny plants from these test crosses should be semisterile.

In order to assure a uniform environment for the interchange event, seed samples of all the desirable combinations from step 4 crosses were alloted to sixteen-foot rows in two connecting oblong blocks with a completely randomized design, and planted on the same date, May 13, 1972. The homogeneity of the soil condition was considered to minimize the possible experimental error.

The Location of the wx Locus and the Length of the Rearranged Chromosome

The precise position of the wx locus in the short arm of chromosome 9 (9S) has never been determined. McClintock (1941) reported that wx is located at approximately the middle of the short arm, and is distal to the dark staining one-third of the arm adjacent to the centromere. It was also placed distal to Ds as illustrated by her photographs and diagram (McClintock, 1951). According to Roman and Ullstrup (1951) the approximate position of the breakage point of TB-9b is 9S.40. Since the wx locus is not uncovered by TB-9b it can be inferred that the cytological distance between wx and the breakage point of such a translocation is quite negligible (Bianchi and Borghi, 1966). Genetic data suggest that the crossover distance is also small; the wx locus is about 0.1 map unit from the breakage point of TB-9b (Bianchi, 1968). Rhoades (personal correspondence)

commented that the wx locus could not be any nearer than 9S.40. From an overall consideration of the above situations, 9S.40 is optionally adopted as the chromosome position of the wx locus in this study.

The length of the rearranged chromosome is determined from the two chromosomes involved in the interchange. For example, the proximal translocation contains wx in the noncentromere 9S segment, distal to the breakage point on 9S, and another chromosome containing a centromere but without a segment of one arm. Thus the whole length of the rearranged chromosome will be the sum of the distal 9S segment plus the main portion of another broken chromosome including its centromere. The distal translocation chromosome contains the long arm and proximal portion of the short arm (including wx) of chromosome 9 as well as a distal segment from another chromosome. The physical length (in micron) of maize chromosomes and their arm ratios are according to Rhoades (1950), and the position of breaks are from Longley (1961). The arm ratio of chromosome 6 has been confirmed to be 3.1:1.0 (Maguire, 1962; Rhoades and Dempsey, personal correspondences), although Rhoades (1950), Neuffer et al. (1968), and W. L. Brown (unpublished notes) gave ratios of 7.1, 7.0, and 6.2:1.0 for this chromosome, respectively.

Pollen Collection and the Recombination Assay

Tassel segments to be used in the pollen assay are collected the day before anthesis and include the tassel segment from the main spike of the tassel adjacent to freshly shed anthers. These are stored in 70 percent ethanol before staining. A curing period of several weeks is desirable. The solution used to stain pollen is a modification of that suggested by Nelson (1968). The formulation for the stain is 25 ml H_2O, 250 mg KI and 45 mg I_2. The KI is dissolved in a minimal quantity of water necessary for solubilizing and then the I_2 added before diluting to total volume. One drop of Tween 80 is stirred into the I_2-KI solution before mixing with 0.3 gram of the Baker's gelatin. The gelatin is solubilized by heating on a hot plate for 5 minutes. Fresh stain is prepared weekly during periods of use.

In preparing the slide about 60 anthers (3 anthers from each of 20 florets) are picked, placed on the surface of a 3.25" x 4" lantern slide (Esco groundedge cover glass) containing several drops of stain. The anthers are then cut apart and gentle pressure is applied in order to release the pollen grains into the solution. After removing all the debris, the pollen grains are distributed evenly (adding additional stain if necessary) and a 50 x 75 mm cover slip (Corning cover glass) is applied to the slide. The preparation is ready for counting, and also exhibits the maximum differential staining at this

time. There are more than 100,000 pollen grains on one slide. The slide can be stored a few days by coating the edges of the cover slip with colorless nail polish. However, if the stain is not well prepared, the stained pollen grains will become less distinguishable after a period of time.

An AO Cycloptic binocular (Stereoscopic Microscope, Series 58) with adequate illumination from under the slide is used to scan the pollen grains. The estimation of the total pollen population on the slide is obtained by multiplying the average of 10 counts from the randomized sites on the slide by a "constant", 940.

The grid in the eyepiece covers 4 mm^2 (2 mm x 2 mm) on the glass stage of the binocular (when magnification is set at 25X) which is 1/937.5 (4 mm^2/3750 mm^2) of the total area of the cover slip (3750 mm^2 = 50 mm x 75 mm). Since pollen grains distributed on the edge lines of the control area are likely to be counted, 940 has been used in order to compensate for this minor bias.

RESULTS

Four wx alleles and ten translocations, five proximal (T1-T5) and five other of a distal series (T6-T10) are used in this study. With four wx alleles C, B, 90 and H21 there are six possible heteroallelic combinations, C/B, C/90, C/H21, B/90, B/H21 and 90/H21. With five proximal chromosome interchanges (breaks on chromosome proximal to wx) thirty combinations are possible. In the distal series (breaks distal to wx) one B/90 and four C/90 heteroallelic combinations are tested on five chromosome interchanges. Of these 35, eighteen are completed and reported here (Table 2). Included are thirteen of the proximal series and five of the distal series.

Data on the frequency of Wx in wx^x/wx^y stocks are obtained from several allelic combinations; homoallelic standard chromosome, heteroallelic standard chromosome, heteroallelic homotranslocation chromosome, and homoallelic homotranslocation chromosome. The homoallelic standard serves as a general control. The Wx frequency values of the four wx homoalleles on the standard chromosome, both from the inbred source and from the crosses of waxy heterozygous parents, are shown in Table 3. The recombination frequencies of 6 heteroallelic combinations at the wx locus of the standard chromosome (controls) and 18 heteroallelic combinations on interchanged chromosomes (treatments) are shown in Table 4. The Wx frequencies of those homoallelic homotranslocations are shown

Table 2. The possible heteroallelic homotranslocation combinations (-) and the recovered lines (X)

Proximal	T1	T2	T3	T4	T5	Control
C/B	X	X	-	X	X	X
C/90	X	-	-	X	X	X
C/H21	-	X	-	-	-	X
B/90	X	-	-	X	X	X
B/H21	-	X	X	-	-	X
90/H21	-	-	-	-	-	X

Distal	T6	T7	T8	T9	T10	Control
B/90	X					X
C/90		X	X	X	X	X

in Table 5. In each case, over one-half million pollen grains for each combination (Tables 3 and 5, column 4; Table 4, column 5) from five plants are sampled to obtain the wx recombination results.

Comparison of the Wx Frequencies between Inbred and Outcross Sources of Parental Homoalleles on Standard Chromosomes

For the parental stocks there are low, but measurable, frequencies of Wx pollen grains. This varies between lines from the inbred sources (self-crossed or sib-crossed) and

Table 3. The Wx frequencies of four parental homoalleles on standard chromosomes, both from inbred parents (A) and outcrossed parents (B)[a]

	Line 1	Allele 2	Wx frequency ($\times 10^{-5}$) 3	Estimated no. of gametes ($\times 10^{3}$) 4	$s_{\bar{x}}$[b] 5
A	1	C/C	0.95	633	0.07
	2	B/B	0.77	647	0.10
	3	90/90	0.29*	680	0.18
	4	H21/H21	1.15	609	0.58
B	5	C/C	0.97	615	0.37
	6	B/B	1.54	651	0.83
	7	90/90	3.86*	596	0.96
	8	H21/H21	3.07	651	0.51

[a]Inbred parents = N-$\underline{wx}^{x}$/N-$\underline{wx}^{x}$ x N-$\underline{wx}^{x}$/N-$\underline{wx}^{x}$, outcrossed parents = N-$\underline{wx}^{x}$/N-$\underline{Wx}$ x N-$\underline{wx}^{x}$/N-$\underline{Wx}$'.

[b]$s_{\bar{x}} = (s^2/n)^{\frac{1}{2}}$, standard error; where "s^2" is the sample variance and "n" is the number of observations in the mean.

*Difference is significant at .05 level (t-test, for the difference of the same homoallele from two parental sources).

from the outcrosses (N-$\underline{wx}^{x}$/N-$\underline{Wx}$ x N-$\underline{wx}^{x}$/N-$\underline{Wx}$'). The value of the reversion rate of any mutant may include back mutation, suppressor mutation, complementation, and even contamination from wind-blown pollen grain lodging in one of the glumes to be sampled.

The frequencies of occurrence of Wx pollen grains among

Table 4. The Wx frequencies of heteroallelic combinations on standard (A) and interchange (B and C) chromosomes of four wx alleles[a]

	Line	Allelic combination	Wx frequency ($\times 10^{-5}$)		No. of gametes sampled ($\times 10^3$)	$s_{\bar{x}}$[c]
			Observed	Adjusted[b]		
	1	2	3	4	5	6
A	1	C/B	41.00	39.75	737	5.43
	2	C/90	88.25	85.84	740	9.76
	3	C/H21	51.85	49.83	627	4.57
	4	B/90	0.88	(-1.82)	793	0.43
	5	B/H21	37.03	35.73	651	5.01
	6	90/H21	17.88	14.42	783	2.40
B	7	T1-C/B	29.43	28.87	751	1.46
	8	T1-C/90	33.84	33.33	632	3.39
	9	T1-B/90	0.52	0.01	769	0.32
	10	T2-C/B	26.92	24.18	773	3.37
	11	T2-C/H21	50.68	49.16	734	2.91
	12	T2-B/H21	28.77	26.12	813	3.20

[a] A = controls, B and C = proximal and distal interchanges.

[b] Adjusted = (observed value) - $(P_1 + P_2)/2$; adjusted for reversion and suppressor mutation (figure with "-" symbol should be counted as "0").

[c] $s_{\bar{x}}$ = standard error.

Table 4. (Continued)

Line 1	Allelic combination 2	Wx frequency ($\times 10^{-5}$) Observed 3	Wx frequency ($\times 10^{-5}$) Adjusted[b] 4	No. of gametes sampled ($\times 10^{3}$) 5	$s_{\bar{x}}$[c] 6
13	T3-B/H21	30.38	27.66	770	4.08
14	T4-C/B	41.84	39.31	722	3.94
15	T4-C/90	3.04	(-0.98)	1,084	0.94
16	T4-B/90	35.57	32.06	827	4.66
17	T5-C/B	33.96	33.00	689	2.31
18	T5-C/90	68.62	67.06	721	10.48
19	T5-B/90	0.71	(-0.34)	707	0.44
C 20	T6-B/90	1.83	0.39	711	0.81
21	T7-C/90	44.55	43.55	631	6.80
22	T8-C/90	49.63	47.40	514	2.87
23	T9-C/90	47.73	46.32	631	4.40
24	T10-C/90	59.15	57.07	561	3.64

Table 5. The Wx frequencies of the four parental homoalleles on the proximal (A) and distal (B) interchange chromosomes

	Line 1	Allelic combination 2	Wx frequency ($\times 10^{-5}$) 3	No. of gametes sampled ($\times 10^{3}$) 4	$s_{\bar{x}}$[a] 5
A	1	T1-C/C	0.57	701	0.45
	2	T1-B/B	0.56	716	0.11
	3	T1-90/90	0.46	864	0.08
	4	T2-C/C	1.61	746	0.42
	5	T2-B/B	3.87	646	1.08
	6	T2-H21/H21	1.43	838	0.69
	7	T3-B/B	1.73	636	0.08
	8	T3-H21/H21	3.72	752	1.55
	9	T4-C/C	3.04	724	0.81
	10	T4-B/B	2.03	541	0.72
	11	T4-90/90	5.00	580	1.12
	12	T5-C/C	1.48	677	0.12
	13	T5-B/B	0.45	660	0.06
	14	T5-90/90	1.65	668	0.49
B	15	T6-B/B	1.26	796	0.43
	16	T6-90/90	1.62	924	0.46
	17	T7-C/C	0.90	557	0.34
	18	T7-90/90	1.10	544	0.10
	19	T8-C/C	2.86	524	1.17
	20	T8-90/90	1.60	561	0.05
	21	T9-C/C	0.79	506	0.09
	22	T9-90/90	2.03	599	0.74
	23	T10-C/C	1.80	611	0.65
	24	T10-90/90	2.35	510	0.72

[a] $s_{\bar{x}}$ = standard error.

the four inbred source homoallelic combinations are from 0.29 to 1.15 x 10^{-5}, and range from 0.97 to 3.86 x 10^{-5} in those lines which are developed from the crosses of waxy heterozygous parents (Table 3). The value of each individual homoallele from the inbred source, in every case, is slightly lower than the one from the cross of heterozygote parents. The value of line N-$\underline{wx}^{90}$/N-$\underline{wx}^{90}$ shows a significant difference (t-test) between the two seed origins (Table 3, column 3, lines 3 vs. 7).

It appears that the variability in genetic background does influence the rate of reversion. All the inbred source stocks have been inbred no less than two generations, so they are more homogeneous. The parental stocks that are developed from crosses of waxy heterozygous parents resulted in higher Wx values, which is possibly a kind of heterosis from the stimulation of the heterogeneous genetic background.

Comparison of the Wx Frequencies between Homoallelic and Heteroallelic Combinations on Standard Chromosomes

In order to compensate for back mutation (reversion), suppressor mutation, and other factors, the Wx frequency from heteroallelic combinations has been adjusted by using the factor $(P_1 + P_2)/2$ shown in column 4 of Table 4. The P_1 and P_2 represent the frequencies of Wx originating from the two homozygous parental stocks (either on standard or on interchanged chromosomes) which are shown in Tables 3-B and 5. The mean of the two parental (P_1 and P_2) Wx frequencies is reduced because

the Wx frequency of the F_1 cross includes both the real recombination between two mutant sites as well as back mutation, and/or other factors as expressed by the parental stocks.

The adjusted recombination frequencies of the heteroallelic combinations on standard chromosome (Table 4-A, column 4, lines 1-6) range from lower (e.g., B/90 combination) than the average Wx frequency of its two parental homoallelic stocks to many fold higher. All the five (out of six) heteroallelic combinations which show higher recombination values than that of the parental mean values are highly significantly different (Table 6). The other heteroallelic combination, B/90, shows a highly significant lower Wx value than the mean value of the parental Wx only because its adjusted figure has been estimated as "0" (the adjusted value is negative, -1.82×10^{-5}, which should be treated as "0"). All the above indicate that those alleles used in the heteroallelic crosses occupy different sites within the wx region in the chromosome.

Genetic maps are condensed graphic representations of the relative distances, expressed in relative recombination frequencies, among the genes in one linkage group, i.e., in a single chromosome. The adjusted Wx frequencies of the heteroallelic combinations (Table 4-A, column 4) are the expression of the recombination events within the wx cistron. Therefore, it should be possible to establish a linear order for the mutants within the region. It appears that the heteroallelic combination C/90 has the highest recombination frequency

Table 6. Comparison of the Wx frequencies (x 10^{-5}) between parental homoallele (average value) and heteroallelic combination (adjusted value) on standard chromosome[a]

Heteroallelic combination	Recombination value	Mean value of the two parents	X^2-test
C/B	39.75	1.26[b]	**[c]
C/90	85.84	2.42	**
C/H21	49.83	2.02	**
B/90	0[d]	2.70	**
B/H21	35.73	2.31	**
90/H21	14.42	3.47	**

[a]Chi-square test (data from Tables 3-B and 4-A).

[b]1.26 = (0.97 + 1.54)/2 (from Table 3-B, lines 5-6).

[c]** = significantly different at .01 level.

[d]Any figure compared with 0 is considered to be **.

(85.84 x 10^{-5}), so wx alleles C and 90 should be the farthest apart among the four alleles in this study. Since the Wx frequencies of both combinations C/H21 and 90/H21 (49.83 and 14.42 x 10^{-5}) are lower than that of C/90, it follows that allele H21 might be located between C and 90.

The very low recombination value between alleles B and 90 (negative, after adjustment) indicates that the B and 90 alleles lie very close to each other, or are overlapping in the cistron. Contrary to expectation, however, the recombina-

tion relationship of these two alleles with the others are not similar. The intervention of B allele causes non-additivity and makes mapping of the wx region difficult. The linear order of the wx alleles deduced from a composite of the data (Table 13-A, lines 1-6) is C-H21-90(B).

All the adjusted recombination values of combination B/90, whether on standard chromosomes (Table 13-A, line 4) or on chromosome interchanges (Table 13-B and C, lines 7, 14, 15) are too low to detect a real difference. For this reason the heteroallelic combination B/90 generally will not be included for comparative purposes.

Comparison of the wx Recombination Values of All Interchanges with the Controls: Heteroallelic Homotranslocation vs. Heteroallelic Standard Chromosomes

Recombination at the wx locus has been studied both in standard and in interchange (homotranslocation) chromosomes. The Wx frequencies of the interchange chromosome combinations, both the proximal and the distal series (Table 4-B and 4-C, column 4), are generally lower than that of the controls (Table 4-A, column 4). This is evident among the data listed in Table 4, column 4, where 13 out of 15 combinations under consideration (lines 14-16 are not included) have values lower than or equal to that of the standard chromosomes. The recombination values of the three combinations C/B, C/90 and B/90 involving T4 (Table 4-B, lines 14-16) are not consistent with

all values of other interchange combinations, so these three lines will not be discussed.

When the recombination values of all interchange groups are compared with their controls, significant differences are evident in the C/B, C/90 (lower), and B/90 (higher) groups (Table 7). Utilizing Dunnett's procedure (Steel and Torrie, 1960) it is evident that the adjusted recombination values of heteroallelic combinations C/B on T2, and C/90 on T1, T7, T8 and T9 are significantly lower than that of controls at .01 level, and T10-C/90 combination is significantly lower at .05 level. The heteroallelic combinations C/H21 and B/H21 on the interchanges T2 and T3 (Table 7, lines 3 and 5) are not significantly different from the controls.

The heteroallelic combination B/90 on the standard chromosome (control) has an adjusted Wx frequency of "zero" (Table 4-A, line 4). Since any figure divided by zero is "infinity", the values in the B/90 interchange group are interpreted to be significantly different (higher) from that of the control (Table 7), although all three figures in the interchanges are extremely small.

Comparison of the Recombination Frequencies of the Same Heteroallelic Combination among Different Interchange Chromosomes

The distribution of the recombination frequencies of a heteroallelic combination among different interchange chromosomes is an independent behavior. Duncan's "new multiple-range

Table 7. Comparison of all Wx frequencies (x 10^{-5}) of the heteroalleles on chromosome interchanges with that of control[a]

Allele	Control	Chromosome interchanges				d' .05	d' .01
C/B	39.75	(T4)[b] -	T5 33.00	T1 28.87	T2** 24.18	10.96	14.99
C/90	85.84	T5 67.06	T10* 57.07	T8** 47.40	T9** 46.32	22.47	29.19
		T7** 43.55	T1** 33.33	(T4) -			
C/H21	49.83	T2 49.16				11.49	17.71
B/90	0[c]	(T4) -	T6** 0.39	T1** 0.01	T5 0	1.68	2.30
B/H21	35.73	T3 27.66	T2 26.12			12.42	17.72

[a]Dunnett's procedure, d' = t(Dunnett)$s_{\bar{d}}$ (data from Table 4, column 4).

[b](T4) figure is not used for comparison.

[c]Any figure compared with 0 is considered to be **.

*Significant difference from control at .05 level.

**Significant difference from control at .01 level.

test" (Steel and Torrie, 1960) provides a method of comparison with all sizes of differences which depend on the closeness of the means after ranking, with the smallest value for adjacent means and the largest for the extremes. Applying this test, the combinations which show significant differences among interchanges are C/B and C/90 heteroallelic groups (Table 8).

The recombination values of the C/90 heteroallelic combination on the relocated positions show highly significant differences between T1 (33.33×10^{-5}) and T5 (67.06×10^{-5}); significant differences are found between T5 and T7, T8, and T9 (43.55, 47.40, and 46.32×10^{-5}, respectively) as well as between T1 and T10 (57.07×10^{-5}) combinations (Table 8). In the C/B heteroallelic group a significant difference is evident between T2 (24.18×10^{-5}), and T5 (33.00×10^{-5}), and no significant difference is found between other interchange pairs.

From the distribution of the Wx frequencies of C/B and C/90 heteroallelic groups in the chromosome interchanges, it appears that the distance between the wx locus and the centromere does influence the rate of recombination. The linear correlation between the wx-centromere distances and the wx recombination frequencies on the rearranged chromosomes, combined values of the C/B and C/90 combinations, is shown by the solid line in Figure 3. It appears that the longest wx-centromere distance of T5 (38.79 u) results in the highest recombination values, and the heteroallelic combinations in shorter wx-

Table 8. Comparison of the Wx frequencies (x 10^{-5}) of the same heteroallelic combination among different homotranslocations[a]

Alleles	Homotranslocation					
C/B	T5 33.00 a	T1 28.87 a b	T2 24.18 b			
C/90[b]	T5 67.06 a	T10 57.07 a b	T8 47.40 b c	T9 46.32 b c	T7 43.55 b c	T1 33.33 c
B/90	T6 0.39 a	T1 0.01 a	T5 0 a			
B/H21	T3 27.66 a	T2 26.12 a				

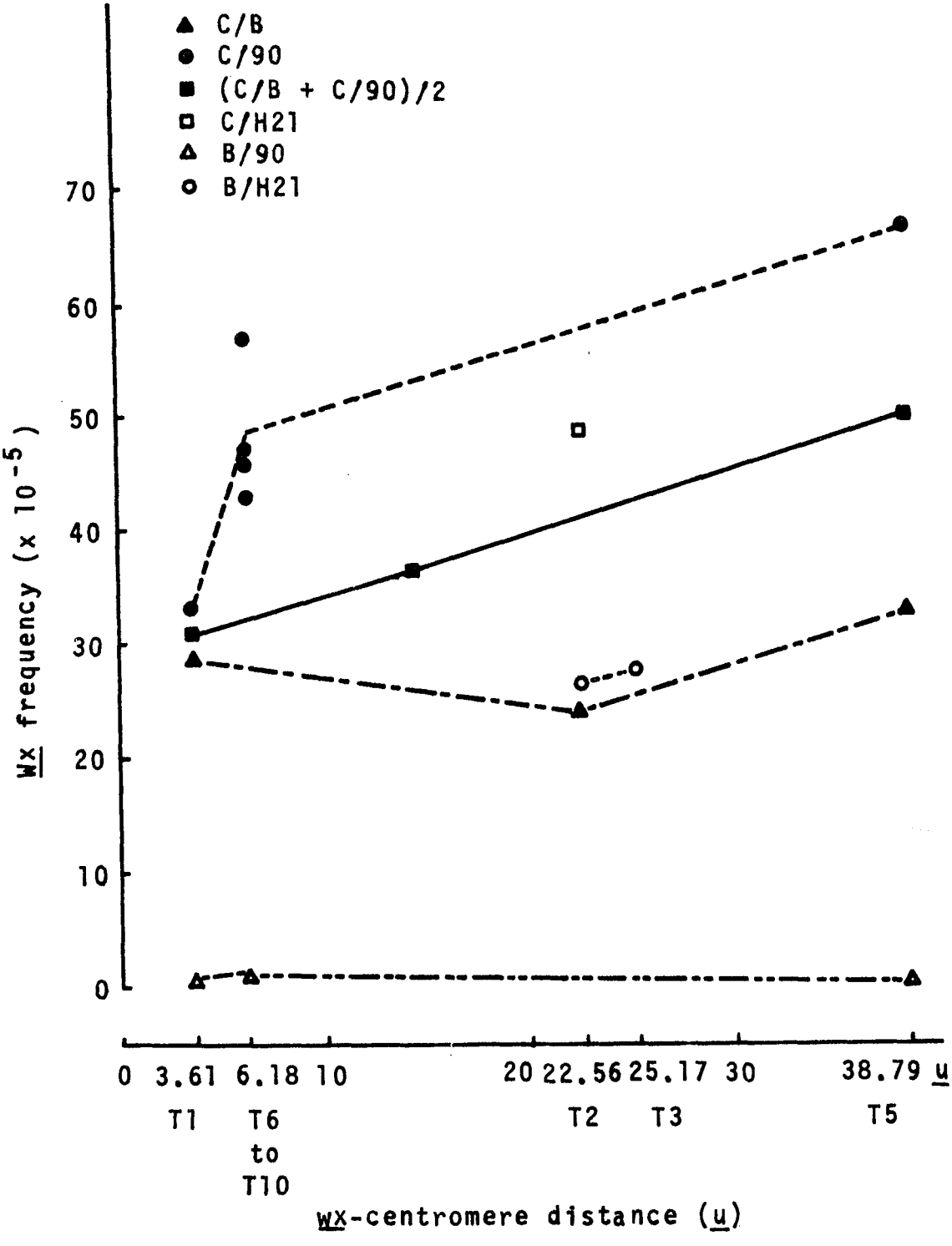
Least significant ranges (LSR)

Value of "p"		2	3	4	5	6
C/B	.05	7.72	8.09			
	.01	10.82	11.40			
C/90	.05	17.21	18.10	18.57	18.98	19.34
	.01	23.35	24.41	25.00	25.53	25.88
B/90	.05	1.74	1.82			
	.01	2.44	2.57			
B/H21	.05	11.96				
	.01	17.39				

[a]Duncan's new multiple-range test on the adjusted data (Table 4, column 4), figures with different letters are significantly different at .05 level; data of T4 group are not included.

[b]Among the interchanges T1 and T5 are significantly different at .01 level.

Figure 3. Test of hypothesis of centromere effect on recombination frequency. Correlation between the wx-centromere distance and Wx recombination on the rearranged chromosomes; wx-centromere distance from Table 1, Wx frequency from Table 4, column 4, lines 7-13 and 17-24 (solid line ——— shows the value for the combined averages of the C/B and C/90 heteroallelic combinations)


C/B
C/90
(C/B + C/90)/2
C/H21
B/90
B/H21
70
60
50
40
30
20
10
0
Wx frequency (x 10⁻⁵)
0
3.61
6.18
10
20
22.56
25.17
30
38.79
u
T1
T6 to T10
T2
T3
T5
wx-centromere distance (u)

centromere distances, i.e., T2, T6-T10, and T1 (22.56 u, 6.18 u and 3.61 u), result in less recombinants (Table 8 and Figure 3).

This linear correlation is also true for the B/H21 combination. Translocations T2 and T3 which have similar wx-centromere distances (22.56 u and 25.17 u) show similar rates of recombination frequencies (26.12 and 27.66 x 10^{-5}, Table 8 and Figure 3). Both interchanges T2 and T3 are involved in the same pair of chromosomes 5 and 9. The B/90 combination does not show a significant difference in Wx frequency among the relocated chromosome positions.

The study of the distal translocation series offers an opportunity to examine the effect of an extended distal segment on wx recombination frequency. There are four distally lengthened segments containing the heteroallelic combination C/90 that can be compared in several ways (Table 4-C, lines 21-24). With Duncan's method, there is no statistical difference among the four adjusted recombination values, although there was a highly significant difference (X^2-test) between the adjusted Wx values of T7 and T10 combinations (55.26 and 94.52 x 10^{-5}) in 1970 (Table 13-C, lines 16-17). This indicates that the nature of the wx locus is not influenced by the changing situation of the distal segment of the chromosome arm, so long as the wx-centromere relationship holds unchanged on the original chromosome 9.

Variation in Percentage Change of the Wx Frequency from Control among Different Heteroallelic Combinations with the Same Translocation

There are four translocations (T1, T2, T4 and T5), and with each there is more than one heteroallelic wx combination available. Among the different heteroallelic combinations for each interchange group, there is a wide range in the reduction of the Wx frequencies from that of the control (Table 9). In view of this arcsine transformation ($\sin^{-1}X^{1/2}$) is applied, since it is especially recommended when the percentages cover a wide range of values (Steel and Torrie, 1960). Also, the statistical analysis requires that the experimental errors be independently and normally distributed with a common variance.

The two heteroallelic combinations C/B and C/90 (B/90 is not included in this comparison) that are relocated to the same T1 position (Table 4-B, lines 7 and 8) show reduced values 27.37% and 61.17% from the controls, which is a highly significant difference between the two lines (Table 9). Three heteroallelic combinations C/B, C/H21 and B/H21 are relocated at T2 (Table 4-B, lines 10-12) and show reduced values of 39.17%, 1.34% and 26.90%, respectively, from the controls, which represents a highly significant difference between C/B and C/H21, and a significant difference between C/H21 and B/H21. With translocation T4 (Table 4-B, lines 14-16), the percentage changes from the controls of C/90 and B/90 combinations are drastic (greater than 100%), hence, no calculation has been

Table 9. Comparison of percentage reduction of the Wx frequency from control among different heteroallelic combinations at the same translocation[a,b]

Interchange	Heteroalleles[c,d]		
T1	C/90 61.17% a	C/B 27.37% b	B/90 (-)
T2	C/B 39.17% a	B/H21 26.90% a	C/H21 1.34% b
T5	C/90 21.88% a	C/B 16.98% a	B/90 (-)

Least significant ranges (LSR)

Value of "p"		2	3
T1	.05	7.24	-
	.01	10.53	-
T2	.05	18.66	19.57
	.01	26.17	27.56
T5	.05	29.86	-
	.01	43.41	-

[a]Duncan's new multiple-range test on the arcsine transformation data; figures with different letters are significantly different at .05 level.

[b]Among the percentages C/90-C/B on T1 and C/B-C/H21 on T2 are significantly different at .01 level.

[c]Data of T4 as well as B/90 combinations are not included.

[d]$61.17\% = \left(1 - \frac{33.33}{85.84}\right) \times 100\%$ (from Table 4, column 4, lines 2 and 8).

done here; their differences are obviously highly significant.

All of the above three interchanges (T1, T2 and T4) which caused significant differences in percentage change of the Wx frequency from controls among different heteroallelic combinations have relatively short wx-breakage point distances (1.08 u, 0.31 u and 0.46 u). In comparison with T5 (Figure 1), which has a longer wx-breakage point distance (4.79 u), no statistical difference in degree of reduction of Wx value from the control between C/B (16.98%) and C/90 (21.88%) was found (Table 9). Therefore, a greater difference in the degree of change of recombination frequencies from controls among heteroallelic combinations at the same relocated position is mainly influenced by the closeness of the wx-breakage point distance; thus, with a shorter wx-breakage point distance, a greater change occurs in allelic recombinations among the relocated segments.

Comparing the Wx Frequencies among the Homoallelic Combinations on Standard and Homotranslocation Chromosomes

For the homoallelic series there are twenty-four combinations among the translocations (Table 5) and four combinations among the controls (Table 3). The mutant homozygotes contain considerable differences in Wx frequencies on the relocated positions. Within each homoallelic group, at least one or more interchange combinations show numerically higher Wx values than that of the standard chromosome (Table 10). This indicates that the new chromosomal position stimulates reversions in the

Table 10. Comparison of the Wx frequencies ($\times 10^{-5}$) of all homoalleles on interchange chromosomes with that of controls[a]

Allele	Control	Interchange chromosomes				d' .05	d' .01
C/C	0.97	T4 3.04	T8 2.86	T10 1.80	T2 1.61	2.42	2.94
		T5 1.48	T7 0.90	T9 0.79	T1 0.57		
B/B	1.54	T2 3.87	T4 2.03	T3 1.73	T6 1.26	2.50	3.09
		T1 0.56	T5 0.45				
90/90	3.86	T4 5.00	T10 2.35	T9 2.03	T5 1.65	2.78	3.36
		T6 1.62	T8 1.60	T7 1.10	T1** 0.46		
H21/H21	3.07	T3 3.72	T2 1.43			3.61	4.89

[a]Dunnett's procedure, $d' = t(\text{Dunnett})s_{\bar{d}}$ (data from Tables 3-B and 5).

**Significantly different from control at .01 level.

new location within genome.

In comparing the values of all interchange combinations with that of controls (Table 10) only the 90/90 group shows a highly significant difference. According to Dunnett's procedure the difference between T1-90/90 (0.46×10^{-5}) and the control (3.86×10^{-5}) is significant. All C/C, B/B and H21/H21 groups show no significant differences between values among all interchange combinations and the controls.

When the same homozygous wx allele is relocated at the different chromosomal positions, there are differences within the C/C, B/B and 90/90 grcups (Table 11). In the 90/90 group the value of T4-90/90 (5.00×10^{-5}) is highly significantly different from all the other combinations. The B/B group shows highly significant differences between T2 (3.87×10^{-5}) and T1, T5, and T6 combinations (0.56, 0.45 and 1.26×10^{-5}), and significant differences between T2 and T3 (1.73×10^{-5}) as well as between T2 and T4 (2.03×10^{-5}). Significant differences are also found in the C/C group between T4 (3.04×10^{-5}) and T1, T7 and T9 (0.57, 0.90 and 0.79×10^{-5}), and between T8 (2.86×10^{-5}) and T1 as well as between T8 and T9 combinations in the same group. The H21/H21 group contains T2 and T3 combinations which do not show a difference between each other. The above results indicated that there is little relationship between the Wx frequency arising from the homoallelic combinations and the wx-centromere or wx-breakage point distance.

The different homoalleles at the same chromosome position

Table 11. Comparison of the Wx frequencies ($\times 10^{-5}$) of the same homoallele among different translocations[a, b]

Allele	Homotranslocation							
C/C	T4 3.04 a	T8 2.86 a b	T10 1.80 a b c	T2 1.61 a b c	T5 1.48 a b c	T7 0.90 b c	T9 0.79 c	T1 0.57 c
B/B	T2 3.87 a	T4 2.03 b	T3 1.73 b	T6 1.26 b	T1 0.56 b	T5 0.45 b		
90/90	T4 5.00 a	T10 2.35 b	T9 2.03 b	T5 1.65 b	T6 1.62 b	T8 1.60 b	T7 1.10 b	T1 0.46 b
H21/H21	T3 3.72 a	T2 1.43 a						

Least significant ranges (LSR)

Value of "p"		2	3	4	5	6	7	8
C/C	.05	1.77	1.87	1.92	1.97	2.00	2.03	2.05
	.01	2.39	2.49	2.56	2.59	2.65	2.68	2.71
B/B	.05	1.72	1.81	1.86	1.90	1.93		
	.01	2.33	2.44	2.50	2.55	2.59		
90/90	.05	1.84	1.94	1.99	2.04	2.07	2.10	2.12
	.01	2.48	2.61	2.65	2.69	2.75	2.78	2.81
H21/H21	.05	3.90						
	.01	5.67						

[a]Duncan's new multiple-range test (data from Table 5, column 3); figures with different letters are significantly different at .05 level.

[b]Among interchanges T1-T2, T2-T5 and T2-T6 in the B/B group as well as T4 with all other interchanges in 90/90 group are significantly different at .01 level.

show some variation in the relative percentage changes of Wx frequencies from the controls. In a comparison of ten interchanges, nine show a high degree of difference (Table 12). The one which does not show a difference is translocation T3, which involves two homoalleles B and H21. Three combinations have their percentage changes greater than 100% from the controls, i.e., T2-B/B, T4-C/C and T8-C/C (+151.3%, +213.4% and +194.8%). There, arcsine transformation cannot be applied for the analysis; Chi-square test is utilized here. It shows that different homoalleles have great differences in the frequency of reversion. No particular trend was noted from the features of the rearranged chromosomes, neither from the distances of wx-centromere and wx-breakage point nor from the length of the distal segment from the wx locus.

Effect of Environment: The Effect of Different Years on the Wx Reversion Frequencies among Various Allelic Combinations

The Wx frequency that is characteristic of a cross between two wx alleles could be influenced by environmental factors as well as by genetic background. All available data show some level of change in figures among different years (Table 13). Among the heteroallelic combinations on standard chromosomes showing a change in 1970 three combinations involving the C allele increased, and the other combinations decreased. In the 1971 season, all recombination values decreased unidirectionally. In a between year comparison, the greatest changes

Table 12. Percentage changes of the Wx frequency from control among different homoallelic combinations at the same interchange chromosome[a]

Interchange	X^2-test	C/C	B/B	90/90	H21/H21
T1	**[b]	-41.2	-63.6	-88.1	-
T2	**	66.0	151.3	-	-53.4
T3	ns[c]	-	12.3	-	21.2
T4	**	213.4	31.8	29.5	-
T5	**	52.6	-70.8	-57.3	-
T6	**	-	-18.2	58.0	-
T7	**	- 7.2	-	-71.5	-
T8	**	194.8	-	-58.6	-
T9	**	-18.6	-	-47.4	-
T10	**	85.6	-	-31.9	-

[a]Chi-square test (data from Tables 3-B and 5).

[b]**Significantly different at .01 level.

[c]ns = nonsignificant in difference.

from 1969 to 1970 are in C/90 (increasing from 69.60 to 109.99 x 10^{-5}) and in B/H21 (decreasing from 94.20 to 52.11 x 10^{-5}), and these changes are highly significant. In 1971, only the C/B combination shows a highly significant decrease (from 73.79 to 39.75 x 10^{-5}), and the C/H21 and 90/H21 decreased significantly (from 72.59 to 49.83 x 10^{-5} and from 27.89 to 14.42 x 10^{-5}, respectively) from 1970.

Table 13. Comparison of the Wx frequencies ($\times 10^{-5}$) of heteroallelic combinations on standard (A) and interchange (B and C) chromosomes in different years[a]

	Line	Combination	1969[b]	1970	1971[c]
A	1	C/B	63.19	73.79	39.75**
	2	C/90	69.60	109.99**	85.84
	3	C/H21	52.21	72.59	49.83*
	4	B/90	1.33	0	0
	5	B/H21	94.20	52.11**	35.73
	6	90/H21	37.22	27.89	14.42*
B	7	T1-B/90	1.02	0	0.01
	8	T2-C/B	36.52	27.48	24.18
	9	T2-C/H21	48.17	40.87	49.16
	10	T2-B/H21	47.79	28.31*	26.12
	11	T3-B/H21	41.41	28.63	27.66
	12	T5-C/B	-	45.06	33.00
	13	T5-C/90	-	68.86	67.06
	14	T5-B/90	-	0	0
C	15	T6-B/90	-	0	0.39
	16	T7-C/90	-	55.26	43.55
	17	T10-C/90	-	94.52	57.07**

[a]A = controls; B and C = proximal and distal interchanges.

[b]Unadjusted data of 1969.

[c]From Table 4, column 4.

*Wx frequency significantly changed from the previous year at .05 level.

**Wx frequency significantly changed from the previous year at .01 level.

Among the chromosome interchanges all the 1970 recombination values, where the corresponding data of the previous season is available, show a decrease from 1969. In 1971, however, there are three combinations (T1-B/90, T2-C/H21 and T6-B/90) which increased and the rest decreased from 1970 in recombination frequencies (Table 13). The recombination frequencies of T2-B/H21 decreased significantly (from 47.79 to 28.31 x 10^{-5}) in 1970, and T10-C/90 declined sharply from 94.52 to 57.07 x 10^{-5} in 1971.

Table 14 sums up all results obtained from 1969 to 1971 of both homoallelic and heteroallelic combinations. Among heteroallelic combinations a comparison of the Wx values of all interchanges with the controls shows significant difference in most cases. Both the C/B and C/90 groups showed highly significant differences (lower) in 1970 and 1971, while C/H21 group showed highly significantly different in 1970 only. Values of B/H21 on interchanges which did not show differences from control in 1971 did show highly significant differences in the previous years. The B/90 group shows highly significant differences (higher) in recombination in 1971 only because the control value is zero. The actual recombination values in T1, T5 and T6 are 0.01, 0.00 and 0.39 x 10^{-5}, respectively (Table 13, lines 7, 14 and 15), which represents the lowest figures in the interchange series.

The above results indicate that the frequencies of wx intragenic recombination of heteroalleles on the rearranged

Table 14. Comparison of the Wx frequencies of homoallelic and heteroallelic combinations on the same as well as the different homotranslocations in different years

	Pattern of comparison	Cross	Heteroalleles 1969	Heteroalleles 1970	Heteroalleles 1971	Homoallele 1971
A.	Control vs. interchanges	C/B	-	**[a]	**	-
		C/90	-	**	**	-
		C/H21	ns[b]	**	ns	-
		B/90	ns	ns	**	-
		B/H21	**	**	ns	-
		C/C	--	-	-	ns
		B/B	-	-	-	ns
		90/90	-	-	-	**
		H21/H21	-	-	-	ns
B.	Same alleles at different interchanges[d]	C/B	-	*[c]	*	-
		C/90	-	**	**	-
		B/90	-	ns	ns	-
		B/H21	ns	ns	ns	-
		C/C	-	-	-	*
		B/B	-	-	-	**
		90/90	-	-	-	**
		H21/H21	-	-	-	ns
C.	Different alleles on the same interchange	T1	-	-	**	**
		T2	**	ns	**	**
		T3	-	-	-	ns
		T4	-	-	**	**
		T5	-	ns	ns	**
		T6	-	-	-	**
		T7	-	-	-	**
		T8	-	-	-	**
		T9	-	-	-	**
		T10	-	-	-	**

[a] **Significantly different at .01 level.

[b] ns = nonsignificant in difference.

[c] *Significantly different at .05 level.

[d] Data of T4 as well as C/H21 combinations are not included.

chromosomes are generally lower than that of the control. However, this is not the case for the homoallelic combinations. Ten out of 24 combinations on interchanges have higher reversion values than that on standard chromosomes (Table 10). Only the 90/90 group contains a significant difference in reversion values between all interchanges and the control (Table 14-A).

For the allelic combinations in interchanges two types of comparison can be made; namely, the same heteroalleles at different translocations and different heteroalleles at the same relocated position. In comparing the same heteroallelic combination at different translocations, every heteroallelic group shows a different level of variation. For example, C/90 shows highly significant differences in Wx frequencies among different translocations, and the C/B group shows a difference only at a significant level in both 1970 and 1971 (Table 14-B). Recombination values of B/90 and B/H21 show no differences in the consecutive years. It has been pointed out previously that the only two B/H21 combinations recovered are of T2 and T3, which are involved in the same 5-9 chromosome interchanges. These two interchanges are also represented by similar wx-centromere distances (22.56 u and 25.17 u) as well as similar chromosome lengths (60.31 u and 62.92 u; Table 1). For B/90 combinations, however, the differences among the adjusted values are always too low to detect. Either of these possibilities might be the cause of

the nonsignificant differences among the B/H21 and B/90 interchange combinations. Considering the chromosome constitution and the distribution of Wx frequencies, the distance between the wx locus and centromere seems to be the main reason for the variation of the values among the same heteroallele. This has been shown by the linear correlation in Figure 3.

A comparison among the same homoallele at different translocations also shows different levels of variation. There are highly significant differences in the B/B and 90/90 groups, and a significant difference in the C/C group (Table 14-B). The H21 homoallele relocated at T2 and T3 shows no significant difference. However, all those interchange homoallelic combinations do not indicate any relationship between the wx-centromere distances and the variation of recombination values.

Different wx alleles occupy different mutant sites in the wx cistron, so different single crosses produce different wx recombination frequencies. For this reason a comparison among the relative recombination values of different heteroalleles at the same relocated position has to be done by comparing the relative change in percentages from their respective controls. In heteroallelic combinations this comparison shows highly significant differences at interchanges T1, T2 and T4, but shows no significant difference at the T5 location for consecutive years (Table 14-C). The reduction in the percentages of the T2 group, which do not show a difference in 1970, have

highly significant differences in both 1969 and 1971.

In view of the reconstructed chromosome constitutions, the wx-breakage point distance does influence the variation of percentage changes in recombination values from the controls among different heteroalleles at the same position. The physical wx-breakage point distances of all T1, T2, and T4 interchange chromosomes are shorter than 1.1 u, whereas T5 has a longer distance of 4.8 u (Table 1). This phenomenon is, however, not true for homoalleles at different positions. The wx-breakage point distance does not influence the change in Wx reversion rate from the control among the different homoalleles at the same position.

DISCUSSION

Development of Waxy-Translocation Lines

It takes five steps to develop the desirable genotypes and to obtain pollen grains, both homoallelic and heteroallelic combinations, for the intragenic recombination study. But for various reasons, several chromosomal strands in the proximal series have not been recovered.

There are at least two reasons, namely, the rarity of recoverable crossovers in the step 2 cross and the imitative semisterility in every step. The latter reason caused the pseudo identification of chromosomal strands and, as a consequence, led to making the wrong interchange combinations in subsequent crosses.

In the proximal series of the four wx alleles involved in five interchanges, only 14 out of the possible 20 wx-T chromosome strands have been isolated (Table 5-A, lines 1-14). From these 14 isolated lines only 13 heteroallelic combinations (Table 4-B, lines 7-19) are produced, instead of the 30 combinations if all chromosomal strands are recovered. Besides, single wx alleles involved in one other proximal translocation (T3-9 5775-H21/H21) and three distal translocations (T5-9 6057-C/C, T5-9 8457-90/90, T4-9 6054-C/C and/or -90/90) have been isolated. Thus, heteroallelic combinations on each specific chromosome interchange cannot be made. For that reason those isolated lines are not considered in this study.

During tassel collection (step 5) any heteroallelic combination line with semisterility is avoided because it is assumed to be from an incorrect cross and it follows that one of the two parental lines is possibly without a translocation or with a wrong translocation. For example, the isolated lines T4-9 6054-$\underline{wx}^{C}$/N-$\underline{Wx}$ and -$\underline{wx}^{90}$/N-$\underline{Wx}$ have been confirmed for semisterility after step 4, but their heteroallelic cross T4-9 6054-C/90 progeny did not yield nonabortive pollen grains in 1971. In this particular cross, misjudgment of semisterility on one of the parental plants in the 1970 winter plantings is likely; only one-half of the step 3 progeny plants are expected to be partially abortive during the intercrossing of step 4.

It has been previously noted (Table 4-B, lines 15-16) that the recombination values of combinations T4-C/90 and T4-B/90 (0.00 and 32.06 x 10^{-5}) are not consistent with all values of other interchange combinations. These figures are in contrast to their respective controls, i.e., 85.84 x 10^{-5} for C/90 and 0.00 for B/90 controls. Since pollen from all those plants are not abortive, the homozygosity of interchange T4 is confirmed. Another combination, T4-C/B, however, has an adequate recombination rate 39.31 x 10^{-5} (Table 4-B, line 14). Therefore, a contamination of T4-$\underline{wx}^{C}$ pollen into the T4-$\underline{wx}^{90}$ strain during the crossing of step 3, or a mixture of a T4-$\underline{wx}^{C}$/N-$\underline{Wx}$ kernel into the T4-$\underline{wx}^{90}$/N-$\underline{Wx}$ seeds at step 4 is possible. Other possibilities include the mis-coding of the packet

numbers or mis-seeding to the wrong rows in the field at the process of step 5 planting. In order to check the possible contamination of wx allele 90 from C, the source seeds of T4-C/90 and T4-B/90 combinations are being tested with the homozygous T4-C/C lines. The recombination values of the progeny plants from those crosses should make it possible to detect the identity of the individual alleles involved.

Translocations in maize show differences in degree of pollen abortion and in the appearance of the aborted pollen grains. The differences are also related to the length of the interchanged segments as well as the relative frequencies of different kinds of segregation. The visible pollen abortion in this study is usually clear enough to tell at a glance by a field-scope, i.e., approximately 50% aborted. But in some translocations, e.g., T5 (4L.94; 9S.09), the aborted pollen grains of all combinations available are around 25%, while most crosses in the distal series, less than 40%. It appears that a portion of the adjacent-1 segregation (i.e., homologous centromeres pass to opposite poles) gametes can function when one of the interchanged end segments is relatively short, or dispensable. The explanation of this haplo-viable deficiency-duplication (Phillips, Burnham, and Patterson, 1971) behavior is that the genes in one of the interchanged terminal segments are not essential for production of viable female gametophytes and normal-appearing pollen.

Often the field confirmed semisterility was not expressed

in the progeny generation. For example, T3-$\underline{wx}^{C}$/N-$\underline{Wx}$ (a step 3 plant) was identified in the 1970 summer at the Research Center, but no semisterile plants were found among the progeny plants in Florida. That particular summer plant that caused abortion might be a kind of physiological aberrant. Another example was found in T3-9 5775-$\underline{wx}^{C}$/N-$\underline{Wx}$ in 1968, in which a portion of the tassel was semisterile and the rest of the parts were completely normal. Both types of pollen were crossed to a normal (N) inbred line but all those progeny plants were normal. One incorrect translocation also was found. When the isolated T2-$\underline{wx}^{90}$ chromosome was crossed to other chromosomes with the same translocation but with other $\underline{wx}$ alleles, only the unexpected semisterile progenies were produced. The frequency of aborted pollen grains was approximately 60%, and this indicates that an incorrect translocation (other than T2) for the T2-$\underline{wx}^{90}$ strand is most likely.

Crosses from either direction (as male or female) are considered to be the same for the seed sources, since only haploid pollen grains are used in the analysis. The genotypic background of the crosses are similar, but not necessarily the same for all parental lines. It has been most desirable to keep lines used for crosses as similar as possible.

Analysis of the $\underline{wx}$ Intragenic Recombination

The use of the pollen grain for genetic study has a unique advantage. Their haploid nature makes possible a more critical isolation and examination of the action of specific alleles

without the confounding effects of dominance (Pfahler and Linskens, 1970). Therefore, the chemical reaction of iodine solution on pollen starch is an autonomous expression of the wx allele that is part of the pollen grain genotype.

The term reddish-brown actually includes waxy starches which stain yellow, brown, red, light purple and intermediate shades depending on the condition of starches and iodine solution used. Freshly prepared iodine potassium iodide solution stains denser than older solutions. Under the same conditions of staining, however, a rather sharp contrast is provided in dark-blue or purple-black color imparted by nonwaxy starch grains. Occasionally, partial blue-black pollen grains are observed. Since the criterion of distinction of the wx and Wx starches is a quality of macromolecular constitution, this kind of pollen grain has been classified as Wx.

More than a half-million pollen grains are scored in order to measure the average wx recombination frequency in each line. Contamination of foreign pollen is not likely. Tassel samples have been soaked in ethanol for several weeks, and the bottles have been transported several times before pollen assay is initiated. Any pollen attached on the tassel should be detached during that period of time. In the laboratory only anthers are picked out with forceps from florets to make the preparations. Therefore, any pollen grain lodging in one of the glumes will not be included in the preparation.

The variance used in the statistical analysis is obtained

from the Wx frequency of individual observations, i.e., from the unadjusted values. Every comparison is preceded by the analysis of variance, F-test. It has been consistent that the group that shows significant differences in F-test (at least at .10 level) also shows significant differences in the further tests. In the comparison of all possible pairs of values in the same group in Tables 8, 9, and 11, L.S.D. (least significant difference) is a less critical test than Duncan's new multiple-range test; therefore, Duncan's method is applied.

Characteristics of the wx Alleles

Interallelic complementation has been observed in various organisms including Drosophila, fungi, bacteria and bacteriophage (Fincham, 1966). Recently two complementation groups have been found at the sh_1 locus on maize chromosome 9; the complementation at the phenotypic level is complete, as indicated by the occurrence of plump, nonshrunken kernels in the F_1 hybrid (Chourey, 1971). In this study, however, no Wx kernel was ever found among the heteroallelic crosses of different wx alleles on either standard or interchange chromosomes. Therefore, from the observations of this study, there is no interallelic complementation among the wx alleles tested.

It is possible that the physical basis for the size of these wx mutations is different in separate instances. Deficiencies and inversions of varying sizes could account for mutations that appear to cover a segment of the locus. All

four alleles tested revert at low rates (Table 3) which would tend to discount deficiencies unless a suppressor mutation at another locus produces a gene epistatic to wx is invoked (Nelson, 1968). But the identity of such a locus has not been reported. It appears that inversions as well as base substitutions in DNA polynucleotides, which was suggested by Bianchi and Tomassini (1965), provide better explanations for the wx mutants.

The Position of the wx Locus and the wx Intragenic Recombination

On testing the recombination values in different locations, heteroallelic combinations show a tendency of higher recombination frequencies for the longer distance from the centromere to the wx locus than that of the shorter distance (Table 8 and Figure 3). The centromere effect is obvious. It appears that this effect is evident in the wx intragenic recombination just as it is in intergenic recombinations in a chromosome arm. This is in agreement with Beadle's (1932) and Graubard's (1932) hypothesis that a change in recombination frequency between two genes is a property of the position of the genes in relation to the centromere, and not a function of the genes themselves. These experimental results provide additional evidence that heredity factors, the classical "genes", are ordered in a one dimensional array in chromosomes, divisible by genetic recombination.

In this study, the adjusted intragenic recombination values of the heteroallelic homotranslocation combinations are usually (except the B/90 group) lower than that of controls (Table 7). It seems that the reunion of chromosomal segments (both in the proximal and distal series) might lessen the tightness of synapsis during meiotic prophase. For the same translocation different allelic interchange chromosomes are isolated from chromosome strands of different crosses, so that their three dimensional breakage-rejoined topographies differ individually. The heteroallelic homotranslocation chromosomes are, therefore, virtually a pair of heteromorphic homologues. This fact is reflected by the Wx values of the homoallelic homotranslocation combinations. The Wx values of interchange combinations of these homomorphic homologues (their synapsis is tighter) are rated higher and lower around that of controls; 42% (10/24) of the interchange combinations show a higher value than the control (Table 10).

Influence on the tightness of synapsis can also be shown in another aspect, that is the effect of the distance between the wx locus and the breakage point. In a comparison of percentage reduction of the Wx frequency from control among different heteroallelic combinations at the same translocation, the relatively short wx breakage point distances caused a greater diversity in reduction rates.

Tightness of synapsis in the vicinity of the breakage point is dependent on the similarity of physical topography at

the point of reunion. For this reason, a locus farther away from the breakage point would be less affected by this perturbation. All the recovered combinations which involved T5 (an interchange with the longest wx-breakage point distance in addition to the longest wx-centromere distance in the proximal series) resulted in more similar rates of Wx values to that of the control than any other heteroallelic combinations in the same groups (Table 7).

The wx Intragenic Recombination Study and Its Possible Extension

Association of characters in inheritance due to the location of genes in close proximity on the same chromosome is another expression for linkage. Linkage may be an aid or a hindrance to gene recombination. A plant breeder's task in a crossing program is to identify a favorable gene (or genes) in a particular accession, dislodge this gene from its neighboring genes and thereby introduce it to the elite lines (Peterson and Yu, 1972). In rare cases two genes conditioning two desirable characters and appearing to be allelic, but available only in separate stocks, could be combined by means of intra-allelic recombination (Burnham, 1966). Similarly two genes conditioning two desirable characters that appear to be nonallelic, and are available only in separate stocks, could be combined by means of intergenic recombination. Difficulties in linkage relationship are observed when favorable and unfavorable

genes alternate closely on the chromosome.

In this study the analysis of intragenic recombination phenomenon at the wx locus is not the total objective. The wx locus is used as a tool to investigate the diversity of the recombination potential within a genome, i.e., in different parts of the maize genome. The results (e.g., the linear correlation between the wx-centromere distance and the wx recombination rate on the rearranged chromosomes; Figure 3) indicate that the success of dislodging a desirable gene and introducing it to the more favorable lines is dependent on the specific location of this particular gene within the chromosome. Gene linkage break-up is dependent on the gene position. It is evident that each gene must be considered individually since the possibility of developing a desirable linkage combination is dependent on the specific location of the gene on the chromosome.

SUMMARY AND CONCLUSIONS

This is a study of the recombination potential in various locations of the maize genome facilitated by an efficient monitoring system, intragenic recombination at the waxy locus. Utilizing the chromosome translocation method, the waxy (wx) locus on chromosome 9 has been relocated to various positions in the maize genome. The four wx alleles used in this recombination study were wx^{C}, wx^{B}, wx^{90}, and wx^{H21}. Two types of translocations were utilized to influence the wx locus. The proximal translocation stocks (T1-T5) were crossed to all the four alleles to move the wx locus to various positions away from the centromere (other than chromosome 9). In the distal series one translocation (T6) was crossed to wx^{B} and wx^{90}, and the other four (T7-T10), to alleles wx^{C} and wx^{90}. These crosses were used to lengthen the short arm of chromosome 9 with a segment from another chromosome of the same genome. A total of twenty-four waxy translocation chromosome strands (14 in proximal and 10 in distal series) were isolated, and therefore, 18 possible heteroallelic combinations were obtained for this intragenic recombination study.

For the parental homoallelic stocks on the standard chromosome there were low, but measurable frequencies of Wx pollen grains produced. The frequency of Wx of each individual homoallele (i.e., wx^{x}/wx^{x}) from the inbred source, in every case, was slightly lower than the one from the cross of waxy

outcrossed parents. It appears that the variability in genetic background can influence the rate of back mutation; this is possibly a kind of heterosis from the stimulation of the heterogeneous genetic background.

The adjusted wx recombination frequencies of the heteroallelic combinations on the standard chromosome ranged from lower (i.e., wx^{B}/wx^{90} combination) than the mean Wx value of its two parental homoalleles to many fold higher. All those differences were statistically highly significant. This indicates that all the four alleles tested are occupying different mutant sites in the wx region in the chromosome. The linear order of these alleles under investigation is wx^{C}-wx^{H21}-wx^{90}(wx^{B}).

In most instances, the frequencies of wx intragenic recombination of the rearranged chromosomes, both in proximal and in distal series, were lower (except the wx^{B}/wx^{90} group) than that of the controls. However, all adjusted recombination values of the heteroallelic wx^{B}/wx^{90} combinations, whether on the standard chromosome or on chromosome interchanges, were too low to detect a real difference.

There was a linear correlation between the wx-centromere distances and the wx recombination frequencies for the combined values of different heteroallelic combinations. The longer distance between the wx locus and centromere resulted in the higher recombination value. All the wx^{C}/wx^{90} combinations that were involved in distal interchanges did not show any

significant difference between each other among their Wx frequencies.

Significant differences in percentage change in recombination value from control were found for different heteroallelic combinations at the same proximal relocated position. This was influenced principally by the closeness of the wx-breakage point distance. A greater diversity in degree of change from control was associated with the shorter distance between the wx locus and the breakage point.

The Wx values of the homoallelic combinations on rearranged chromosomes were rated higher and lower around that of controls. For the same homoallelic combination at different translocations and different homoalleles at the same relocated positions, there were highly significant differences in comparisons within either group. However, no particular trend was found from the feature of the rearranged chromosomes, neither from the distances of wx-centromere and wx-breakage point nor from the length of the distal segment from the wx locus.

In addition to the effects of genetic background the environmental effects were also obvious. The same allelic combination usually resulted in different rates of recombination in different plantings. The change in recombination frequency was either an increase or a decrease.

BIBLIOGRAPHY

Amano, E. 1968. Comparison of ethyl methanesulfonate- and radiation-induced waxy mutants in maize. Mutation Research 5: 41-46.

Amano, E. and H. H. Smith. 1965. Mutations induced by ethyl methanesulfonate in maize. Mutation Research 2: 344-351.

Anderson, E. G. 1925. Crossing over in a case of attached-X chromosomes in Drosophila melanogaster. Genetics 10: 403-417.

Anderson, E. G., H. H. Kramer, and A. E. Longley. 1955a. Translocations in maize involving chromosome 4. Genetics 40: 500-510.

Anderson, E. G., H. H. Kramer, and A. E. Longley. 1955b. Translocations in maize involving chromosome 6. Genetics 40: 531-538.

Badenhuizen, N. P. 1969. The Biogenesis of Starch Granules in Higher Plants. New York, N.Y., Appleton-Century-Crofts, Meredith Corporation.

Ballantyne, G. H. and A. Chovnick. 1971. Gene conversion in higher organisms: Non-reciprocal recombination events at the rosy cistron in Drosophila melanogaster. Genetical Research 17: 139-149.

Beadle, G. W. 1932. A possible influence of the spindle fiber on crossing over in Drosophila. National Academy of Sciences, Proceedings 18: 160-165.

Belling, J. 1933. Crossing over and gene rearrangement in flowering plants. Genetics 18: 388-413.

Benzer, S. 1955. Fine structure of a genetic region in bacteriophage. National Academy of Sciences, Proceedings 41: 344-354.

Benzer, S. 1956. Genetic fine structure and its relation to the DNA molecule. Brookhaven Symposia in Biology 8: 3-5.

Benzer, S. 1957. The elementary units of heredity. In W. D. McElroy and B. Glass, eds. The Chemical Basis of Heredity. Pp. 70-93. Baltimore, Md., Johns Hopkins Press.

Benzer, S. 1959. On the topology of the genetic fine structure. National Academy of Sciences, Proceedings 45: 1607-1620.

Benzer, S. 1961. On the topography of the genetic fine structure. National Academy of Sciences, Proceedings 47: 403-415.

Bianchi, A. 1968. A fine analysis of crossing-over in maize. Proceedings of the XII International Congress of Genetics, Vol. 1: 170.

Bianchi, A. and B. Borghi. 1966. Very low cross-over rate between wx and the breakage point of TB-9b. Maize Genetics Cooperation News Letter 40: 75-76.

Bianchi, A. and C. Tomassini. 1965. Reversion frequency of waxy pollen type in normal and hypoploid maize plants. Mutation Research 2: 352-365.

Bridges, C. B. 1915. A linkage variation in Drosophila. Journal of Experimental Zoology 19: 1-21.

Bridges, C. B. 1916. Non-disjunction as a proof of the chromosome theory of heredity. Genetics 1: 1-52, 107-163.

Bridges, C. B. and K. S. Brehme. 1944. The Mutants of Drosophila melanogaster. Carnegie Institution of Washington Publication 552.

Briggs, R. W. 1968. Genetic recombination among spontaneous and ethyl methanesulfonate (EMS) induced waxy mutants in maize. Maize Genetics Cooperation News Letter 42: 16-19.

Briggs, R. W. and H. H. Smith. 1965. Effects of X-radiation on intracistron recombination at the waxy locus in maize. Journal of Heredity 56: 157-162.

Briggs, R. W., E. Amano, and H. H. Smith. 1965. Genetic recombination with ethyl-methanesulphonate-induced waxy mutants in maize. Nature 207: 890-891.

Brink, R. A. 1929. An enzyme difference associated with the waxy gene in maize. Genetics 14: 569-590.

Brink, R. A. and J. H. MacGillivray. 1924. Segregation for the waxy character in maize pollen and differential development of the male gametophyte. American Journal of Botany 11: 465-469.

Brown, M. S. 1940. The relation between chiasma formation and disjunction. University of Texas Publication 4032: 11-64.

Burnham, C. R. 1932. The association of non-homologous parts in a chromosomal interchange in maize. Proceedings of the Sixth International Congress of Genetics, Vol. 2: 19-20.

Burnham, C. R. 1934. Chromosomal interchanges in maize: reduction of crossing over and the association of non-homologous parts. American Naturalist 68: 81-82.

Burnham, C. R. 1962. Discussions in Cytogenetics. Minneapolis, Minn., Burgess Publishing Company.

Burnham, C. R. 1966. Cytogenetics in plant improvement. In K. J. Frey, ed. Plant Breeding. Pp. 139-187. Ames, Iowa, Iowa State University Press.

Carter, J. H. and E.Y.C. Lee. 1971. An enzymic method for determination of the average chain lengths of glycogens and amylopectins. Analytical Biochemistry 39: 373-386.

Chourey, P. S. 1971. Interallelic complementation at the Sh_1 locus in maize. Genetics 68: 435-442.

Chovnick, A., G. H. Ballantyne, D. L. Baillie, and D. G. Holm. 1970. Gene conversion in higher organisms: Half-tetrad analysis of recombination within the rosy cistron of Drosophila melanogaster. Genetics 66: 315-329.

Cooper, K. W., S. Zimmering, and J. Krivshenko. 1955. Interchromosomal effects and segregation. National Academy of Sciences, Proceedings 41: 911-914.

Creech, R. G. 1968. Carbohydrate synthesis in maize. Advances in Agronomy 20: 275-322.

Creighton, H. B. and B. McClintock. 1932. Cytological evidence for 4-strand crossing over in Zea mays. Proceedings of the Sixth International Congress of Genetics, Vol. 2: 392.

Demerec, M. 1924. A case of pollen dimorphism in maize. American Journal of Botany 11: 461-464.

Dobzhansky, T. 1930. Translocations involving the third and fourth chromosomes of Drosophila melanogaster. Genetics 15: 347-399.

Dobzhansky, T. 1931. The decrease in crossing over observed in translocations, and its probably explanation. American Naturalist 65: 214-232.

Dobzhansky, T. and A. H. Sturtevant. 1931. Translocations between the second and third chromosomes of Drosophila and their bearing on Oenothera problems. Carnegie Institution of Washington Publication 421: 29-59.

Esposito, R. E. 1968. Genetic recombination in synchronized cultures of Saccharomyces cerevisiae. Genetics 59: 191-210.

Fincham, J.R.S. 1966. Genetic Complementation. New York, N.Y., W. A. Benjamin, Inc.

Fincham, J.R.S. 1970. Fungal genetics. Annual Review of Genetics 4: 347-372.

Fogel, S. and D. D. Hurst. 1967. Meiotic gene conversion in yeast tetrads and the theory of recombination. Genetics 57: 455-481.

Fogel, S., D. D. Hurst, and R. K. Mortimer. 1971. Gene conversion in unselected tetrads from multipoint crosses. Stadler Symposia Vol. 1 and 2: 89-110.

Fogel, S. and R. K. Mortimer. 1969. Informational transfer in meiotic gene conversion. National Academy of Sciences, Proceedings 62: 96-103.

Foster, J. F. 1943. Characterization of components of starch. Unpublished Ph.D. dissertation, Ames, Iowa, Library, Iowa State College.

Gajewski, W., A. Paszewski, A. Dawidowicz, and B. Dudzinska. 1968. Postmeiotic segregation in locus '46' of Ascobolus immersus. Genetical Research 11: 311-317.

Grant, V. 1958. The regulation of recombination in plants. Cold Spring Harbor Symposia on Quantitative Biology 23: 337-363.

Graubard, M. A. 1932. Inversion in Drosophila melanogaster. Genetics 17: 81-105.

Graubard, M. A. 1934. Temperature effect on interference and crossing over. Genetics 19: 83-94.

Greenwood, C. T. and E. A. Milne. 1968. Starch degrading and synthesizing enzymes: A discussion of their properties and action pattern. Advances in Carbohydrate Chemistry 23: 282-366.

Grell, R. F. 1962. A new hypothesis on the nature and sequence of meiotic events in the female of Drosophila melanogaster. Natural Academy of Sciences, Proceedings 48: 165-172.

Grell, R. F. 1967. Pairing at the chromosomal level. Journal of Cellular Physiology Supplement 1 to vol. 70: 119-146.

Hanson, G. P. 1969. B-chromosome-stimulated crossing over in maize. Genetics 63: 601-609.

Hastings, P. J. and H.L.K. Whitehouse. 1964. A polaron model of genetic recombination by the formation of hybrid-DNA. Nature 201: 1052-1054.

Hixon, R. M. and B. Brimhall. 1968. Waxy cereals and red iodine starches. In J. A. Radley, ed. Starch and Its Derivatives. 4th ed. Pp. 247-281. London, Chapman and Hall Ltd.

Hollander, W. F. 1938. A sex difference in linkage intensity of three autosomal factors in the domestic pigeon. Genetics 23: 24-27.

Holliday, R. 1964. A mechanism for gene conversion in fungi. Genetical Research 5: 282-304.

Hollo, J. and J. Szejtli. 1968. The reaction of starch with iodine. In J. A. Radley, ed. Starch and Its Derivatives. 4th ed. Pp. 203-246. London, Chapman and Hall Ltd.

Kempton, J. H. 1919. Inheritance of waxy endosperm in maize. U.S. Department of Agriculture Bulletin 754.

Kitani, Y. and L. S. Olive. 1967. Genetics of Sordaria fimicola. VI. Gene conversion at the g locus in mutant x wild-type crosses. Genetics 57: 767-782.

Kramer, H. H. and R. L. Whisler. 1949. Quantitative effects of certain genes on the amylose content of corn endosperm starch. Agronomy Journal 41: 409-411.

Levine, R. P. 1968. Genetics. 2nd ed. New York, N.Y., Holt, Rinehart and Winston, Inc.

Lewis, E. B. 1967. Genes and gene complexes. In R. A. Brink, ed. Heritage From Mendel. Pp. 17-47. Madison, Wis., University of Wisconsin Press.

Lindegren, C. C. 1953. Gene conversion in Saccharomyces. Journal of Genetics 51: 625-637.

Lindegren, C. C. 1955. Non-Mendelian segregation in a single tetrad of Saccharomyces ascribed to gene conversion. Science 121: 605-607.

Lindegren, C. C. and G. Lindegren. 1937. Non-random crossing over in Neurospora. Journal of Heredity 28: 104-113.

Lissouba, P., J. Mousseau, G. Rizet, and J. L. Rossignol. 1962. Fine structure of genes in the ascomycete Ascobolus immersus. Advances in Genetics 11: 343-380.

Longley, A. E. 1961. Breakage points for four corn translocation series and other corn chromosome aberrations. U.S. Department of Agriculture, Agricultural Research Service, ARS 34-16.

Maguire, M. P. 1962. Variability in length and arm ratio of the pachytene chromosome of corn. Cytologia 27: 248-257.

Maguire, M. P. 1969. Intrachromosomal crossover increase with distal homologous substitution. Nature 222: 691-692.

Manners, D. J. 1968. The biological synthesis of starch. In J. A. Radley, ed. Starch and Its Derivatives. 4th ed. Pp. 66-90. London, Chapman and Hall Ltd.

Mather, K. and R. Lamm. 1935. The negative correlation of chiasma frequencies. Hereditas 20: 65-70.

McClintock, B. 1931. Cytological observations of deficiencies involving known genes, translocations, and an inversion in Zea mays. Missouri Agricultural Experiment Station Research Bulletin 163: 1-30.

McClintock, B. 1941. The stability of broken ends of chromosomes in Zea mays. Genetics 26: 234-282.

McClintock, B. 1944. The relation of homozygous deficiencies to mutations and allelic series in maize. Genetics 29: 478-502.

McClintock, B. 1951. Chromosome organization and genic expression. Cold Spring Harbor Symposia on Quantitative Biology 16: 13-47.

Melnyczenko, W. I. 1970. The effect of B chromosomes on intragenic recombination. Maize Genetics Cooperation News Letter 44: 203-205.

Meselson, M. 1967. The molecular basis of genetic recombination. In R. A. Brink, ed. Heritage from Mendel. Pp. 81-104. Madison, Wis., University of Wisconsin Press.

Mitchell, M. B. 1955. Aberrant recombination of pyridoxine mutants of Neurospora. National Academy of Sciences, Proceedings 41: 215-220.

Morgan, D. T., Jr. 1950. A cytogenetic study of inversions in Zea mays. Genetics 35: 153-174.

Murray, N. E. 1969. Reversal of polarized recombination of alleles in Neurospora as a function of their position. Genetics 61: 67-77.

Murray, N. E. 1970. Recombination events that span sites within neighbouring gene loci of Neurospora. Genetical Research 15: 109-121.

Nel, P. M. 1971. Studies on the genetic control of recombination in Zea mays. Unpublished Ph.D. dissertation. Bloomington, Indiana, Library, Indiana University.

Nelson, O. E. 1957. The feasibility of investigating "genetic fine structure" in higher plants. American Naturalist 91: 331-332.

Nelson, O. E. 1959. Intracistron recombination in the Wx/wx region in maize. Science 130: 794-795.

Nelson, O. E. 1962. The waxy locus in maize. I. Intralocus recombination frequency estimates by pollen and by conventional analyses. Genetics 47: 737-742.

Nelson, O. E. 1964. Differential crossing over in male and female gametes of plants heterozygous for Dp 9. Maize Genetics Cooperation News Letter 38: 124-126.

Nelson, O. E. 1966. The difference in Wx frequency between male and female gametes from $wx^{Coe}/\overline{wx}^{90}$. Maize Genetics Cooperation News Letter 40: 135.

Nelson, O. E. 1968. The waxy locus in maize. II. The location of the controlling element alleles. Genetics 60: 507-524.

Nelson, O. E. and H. W. Rines. 1962. The enzymatic deficiency in the waxy mutant of maize. Biochemical and Biophysical Research Communications Vol. 9: No. 4.

Neuffer, M. G. and G. Ficsor. 1963. Mutagenic action of ethyl methanesulfonate. Science 139: 1296-1297.

Neuffer, M. G., L. Jones, and M. S. Zuber. 1968. The Mutants of Maize. Madison, Wis., Crop Science Society of America.

Paszewski, A. 1970. Gene conversion: Observations on the DNA hybrid models. Genetical Research 15: 55-64.

Peacock, W. J. 1971. Cytogenetic aspects of the mechanism of recombination in higher organisms. Stadler Symposia Vol. 1 and 2: 123-152.

Peat, S., E. J. Bourne, and S. A. Barker. 1948. Enzymic conversion of amylose into amylopectin. Nature 161: 127-128.

Peterson, P. A. and M. H. Yu. 1972. Gene linkage breakup-dependent on gene position. Dean's Newsletter An-141. (February 1972, in press).

Pfahler, P. L. and H. F. Linskens. 1970. Biochemical composition of maize (Zea mays L.) pollen. I. Effects of the endosperm mutants, waxy (wx), shrunken (sh_2) and sugary (su_1) on the amino acid content and fatty acid distribution. The Theoretical and Applied Genetics 40: 6-10.

Phillips, R. L. 1969a. Recombination in Zea mays L. I. Location of genes and interchanges in chromosomes 5, 6, and 7. Genetics 61: 107-116.

Phillips, R. L. 1969b. Recombination in Zea mays L. II. Cytogenetic studies of recombination in reciprocal crosses. Genetics 61: 117-127.

Phillips, R. L., C. R. Burnham, and E. B. Patterson. 1971. Advantages of chromosomal interchanges which generate haplo-viable deficiency-duplications. Crop Science 11: 525-528.

Pipkin, S. B. 1940. Segregation and crossing over in a 2-3 translocation in Drosophila melanogaster. University of Texas Publication 4032: 73-125.

Plough, H. H. 1917. The effect of temperature on crossing over in Drosophila. Journal of Experimental Zoology 24: 147-209.

Putrament, A., T. Rozbicka, and K. Wojciechowska. 1971. The highly polarized recombination pattern within the methA gene of Aspergillus nidulans. Genetical Research 17: 125-131.

Rhoades, M. M. 1933. An experimental and theoretical study of chromatid crossing over. Genetics 18: 535-555.

Rhoades, M. M. 1941. Different rates of crossing over in male and female gametes of maize. Journal of American Society of Agronomy 33: 603-615.

Rhoades, M. M. 1945. On the genetic control of mutability in maize. National Academy of Sciences, Proceedings 31: 91-95.

Rhoades, M. M. 1950. Meiosis in maize. Journal of Heredity 41: 58-67.

Rhoades, M. M. 1955. The cytogenetics of maize. In G. F. Sprague, ed. Corn and Corn Improvement. Pp. 123-219. New York, N.Y., Academic Press, Inc.

Rhoades, M. M. 1961. Meiosis. In J. Brachet and A. E. Mirsky, eds. The Cell. Vol. III. Meiosis and Mitosis. Pp. 1-75. New York, N.Y., Academic Press, Inc.

Rhoades, M. M. 1968. Studies on the cytological basis of crossing over. In W. J. Peacock and R. D. Brock, eds. Replication and Recombination of Genetic Material. Pp. 229-241. Canberra, Australia, Australian Academy of Science.

Rhoades, M. M. and E. Dempsey. 1953. Cytogenetic studies of deficient-duplicate chromosomes derived from inversion heterozygotes in maize. American Journal of Botany 40: 405-424.

Roman, H. L. 1963. Genic conversion in fungi. In W. J. Burdette, ed. Methodology in Basic Genetics. Pp. 209-246. San Francisco, Calif., Holden-Day, Inc.

Roman, H. L. and A. J. Ullstrup. 1951. The use of A-B translocations to locate genes in maize. Agronomy Journal 43: 450-454.

Russell, W. A. and C. R. Burnham. 1950. Cytogenetic studies of an inversion in maize. Scientific Agriculture 30: 93-111. Original not available for examination; cited in Burnham, C. R. 1962. Discussions in Cytogenetics. Minneapolis, Minn., Burgess Publishing Company.

Senti, F. R. and R. J. Dimler. 1959. High amylose corn-properties and prospects. Food Technology 13: 663-667.

Smith, P. D., V. G. Finnerty, and A. Chovnick. 1970a. Intragenic recombination and gene conversion in Drosophila. (Abstract). Genetics 64: s61.

Smith, P. D., V. G. Finnerty, and A. Chovnick. 1970b. Gene conversion in Drosophila: Non-reciprocal events at the maroon-like cistron. Nature 228: 442-444.

Sprague, G. F., B. Brimhall, and R. M. Hixon. 1943. Some effects of the waxy gene in corn on properties of the endosperm starch. Journal of the American Society of Agronomy 35: 817-822.

Stebbins, G. L. 1950. Variation and Evolution in Plants. New York, N.Y., Columbia University Press.

Steel, R.G.D. and J. H. Torrie. 1960. Principles and Procedures of Statistics. New York, N.Y., McGraw-Hill Book Co., Inc.

Stephens, S. G. 1961a. A remote coincidence. American Naturalist 95: 279-293.

Stephens, S. G. 1961b. Recombination between supposedly homologous chromosomes of Gossypium barbadense L. and G. hirsutum L. Genetics 46: 1483-1499.

Stern, C. 1926. An effect of temperature and age on crossing over in the first chromosome of Drosophila melanogaster. National Academy of Sciences, Proceedings 12: 530-532.

Sturtevant, A. H. 1913. The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. Journal of Experimental Zoology 14: 43-59.

Sturtevant, A. H. 1919. Contributions to the genetics of Drosophila melanogaster. III. Inherited linkage variations in the second chromosome. Carnegie Institution of Washington Publication 278: 305-341.

Sturtevant, A. H. and G. W. Beadle. 1936. The relations of inversions in the X-chromosome of Drosophila melanogaster to crossing over and disjunction. Genetics 21: 554-604.

Swanson, C. P. 1957. Cytology and Cytogenetics. Englewood Cliffs, N.J., Prentice-Hall, Inc.

Thompson, P. E. 1964. Evidence on the basis of the centromere effect in the large autosomes of Drosophila melanogaster. Genetics 49: 761-769.

Vineyard, M. L., R. P. Bear, M. M. MacMasters, and W. L. Deatherage. 1958. Development of "Amylomaize" - corn hybrids with high amylose starch. Agronomy Journal 50: 595-602.

Watson, J. D. 1965. Molecular Biology of the Gene. New York, N.Y., W. A. Benjamin, Inc.

Watson, J. D. and F.H.C. Crick. 1953. Molecular structure of nucleic acids. Nature 171: 737-738.

Weatherwax, P. 1922. A rare carbohydrate in waxy maize. Genetics 7: 568-572.

Whitehouse, H.L.K. 1963. A theory of crossing over by means of hybrid deoxyribonucleic acid. Nature 199: 1034-1040.

Whittinghill, M. 1937. Induced crossing over in Drosophila males and its probable nature. Genetics 22: 114-129.

Yanofsky, C., B. C. Carlton, J. R. Guest, and D. R. Helinski. 1964. On the colinearity of gene structure and protein structure. National Academy of Sciences, Proceedings 51: 266-272.

Yanofsky, C., G. R. Drapeau, J. R. Guest, and B. C. Carlton. 1967. The complete amino acid sequence of the tryptophan synthetase A protein (α subunit) and its colinear relationship with the genetic map of the A gene. National Academy of Sciences, Proceedings 57: 296-298.

Yu, M. H. and P. A. Peterson. 1971. Location-dependent, intragenic recombination in maize. Genetica 42: 368-380.

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